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14. ABSTRACT
Background. Government agencies and expert panels have recognized the need for laboratories capable of analyzing tens of thousands of biological samples per day that have hundreds of times more capability than at present. Objectives/Hypothesis. This project aims to develop a new high speed, high volume (high-throughput) laboratory capability that will be linked in a network and operated by several premier institutions. The automated, networked capability will make us stronger against natural diseases and bioterrorist attacks. Specific Aims. With FY06 (initial year) Congressional appropriations, high-throughput bioagent screening and genotyping systems (with quality controls) will be implemented first. These systems will be housed in laboratory space upgraded to BSL3-enhanced (BSL3e) containment that enables the flow of numerous samples containing highly pathologic avian influenza and other select agents (dual-use). With FY07 (available), FY08 (available) and FY 09 (anticipated) Congressional appropriations, automated culturing and phenotyping systems will be implemented next. Study Design. Because of current public health and national security threats, influenza surveillance and analysis will be the initial focus. Over three years, the project will be expanded to include other biothreat agents, bacterial and/or viral. Relevance. The combination of high-throughput and automated systems will enable processing of tens of thousands of samples and provide critical laboratory capacity. The overall project will facilitate rapid expansion to multiple networked sites.

15. SUBJECT TERMS
Keywords. Influenza; Biosecurity; Select Agents; Real-Time Surveillance; High-Throughput Automation; Standardized Laboratory Network; Actionable Information; Pathomics; Surge Capacity.

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INTRODUCTION

Background. Government agencies and expert panels have recognized the need for laboratories capable of analyzing tens of thousands of biological samples per day that have hundreds of times more capability than at present. **Objectives/Hypothesis.** This project aims to develop a new high speed, high volume (high-throughput) laboratory capability that will be linked in a network and operated by several premier institutions. The automated, networked capability will make us stronger against natural diseases and bioterrorist attacks. **Specific Aims.** With FY06 (initial year) Congressional appropriations, high-throughput bioagent screening and genotyping systems (with quality controls) will be implemented first. These systems will be housed in laboratory space upgraded to BSL3-enhanced (BSL3e) containment that enables the flow of numerous samples containing highly pathologic avian influenza and other select agents (dual-use). With FY07 (available), FY08 (available) and FY 09 (anticipated) Congressional appropriations, automated culturing and phenotyping systems will be implemented next. **Study Design.** Because of current public health and national security threats, influenza surveillance and analysis will be the initial focus. Over three years, the project will be expanded to include other biothreat agents, bacterial and/or viral. **Relevance.** The combination of high-throughput and automated systems will enable processing of tens of thousands of samples and provide critical laboratory capacity. The overall project will facilitate rapid expansion to multiple networked sites.

Keywords. Influenza; Biosecurity; Select Agents; Real-Time Surveillance; High-Throughput Automation; Standardized Laboratory Network; Actionable Information; Pathomics; Surge Capacity.

BODY

Introduction. This Annual report on the "UCLA High Speed, High Volume Laboratory Network for Infectious Diseases: Phase I" pertains to the initial year of our proposed program. It involves work performed by the University of California Los Angeles (UCLA) and Los Alamos National Laboratory (LANL). UCLA serves as the contractor and LANL serves as the subcontractor.

Our program was enabled and supported by a congressionally-directed appropriation in the FY 2006 Department of Defense (DoD) budget. A brief history of our application process is as follows. Our initial application for funding was submitted to USAMRAA on June 16, 2006. It underwent external peer review by the American Institute of Biological Sciences (AIBS) and internal review by the Defense Threat Reduction Agency (DTRA-JSTO-CBM). UCLA received notice of award approval from USAMRAA on December 6, 2006 and, at that time, the investigators were instructed to revise their original budget of $6,000,000 to $5,327,000. Subsequently, UCLA received notice of funding award on March 15, 2007 and a Work for Others (WFO) agreement between UCLA and LANL was completed on June 6, 2007.

Our FY 2006 (initial year) application included the provision for three option years. FY 2007 (option year 1) funding has been appropriated by Congress and we submitted an application for these funds on January 18, 2008. It underwent internal review by the Defense Threat Reduction Agency (DTRA-JSTO-CBM). The investigators were instructed to revise our original budget of $6,000,000 to $5,328,160 on April 2, 2008 and, based on correspondence from USAMRAA, they anticipate receipt of option year 1 funds within the next two months. FY 2008 (option year 2) funding has also been appropriated by Congress and UCLA received formal notification to submit an application for these funds on March 18, 2008. FY 2009 (option year 3) funding is anticipated from Congress. As outlined in our initial application, and shown in Figure 1 below, each of these option years will build on the previous one(s).

Prior to receiving our FY 2006 (initial year) DoD award, UCLA received a $9 million grant from the California Office of Homeland Security (CA OHS) in November 2006 to upgrade additional space to BSL3e specifications on campus, procure an automated bioagent accessioning system, procure an automated biobanking system that can hold 800,000 samples at –80C, and procure 150 or more handheld devices that enable paperless recording of field (epidemiologic) data. This complimentary support greatly enhances the value of DoD's investments and greatly increases high-throughput capacity for influenza research and surge capacity for biological emergencies (see Figure 1).

After receiving our FY 2006 (initial year) award, UCLA also received a $18.9 million contract from the National Institute for Allergy and Infectious Diseases (NIAID) in March 2007 to establish one of six Centers of Excellence for Influenza Research of Surveillance (CEIRS). UCLA serves as the prime and/or contracting institution for the Center. The University of California Davis (UCD), University of Alaska Fairbanks (UAF), Wildlife Conservation Society (WCS), and LANL serve as subcontracting institutions for the Center. Over the next five years (2007 – 2012), the Center will collect and analyze 10,000 domestic and 10,000 non-domestic samples (at least 20,000 total) per year from birds and selected mammals. These Center-related activities will significantly compliment and facilitate large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses (see Figure 1).
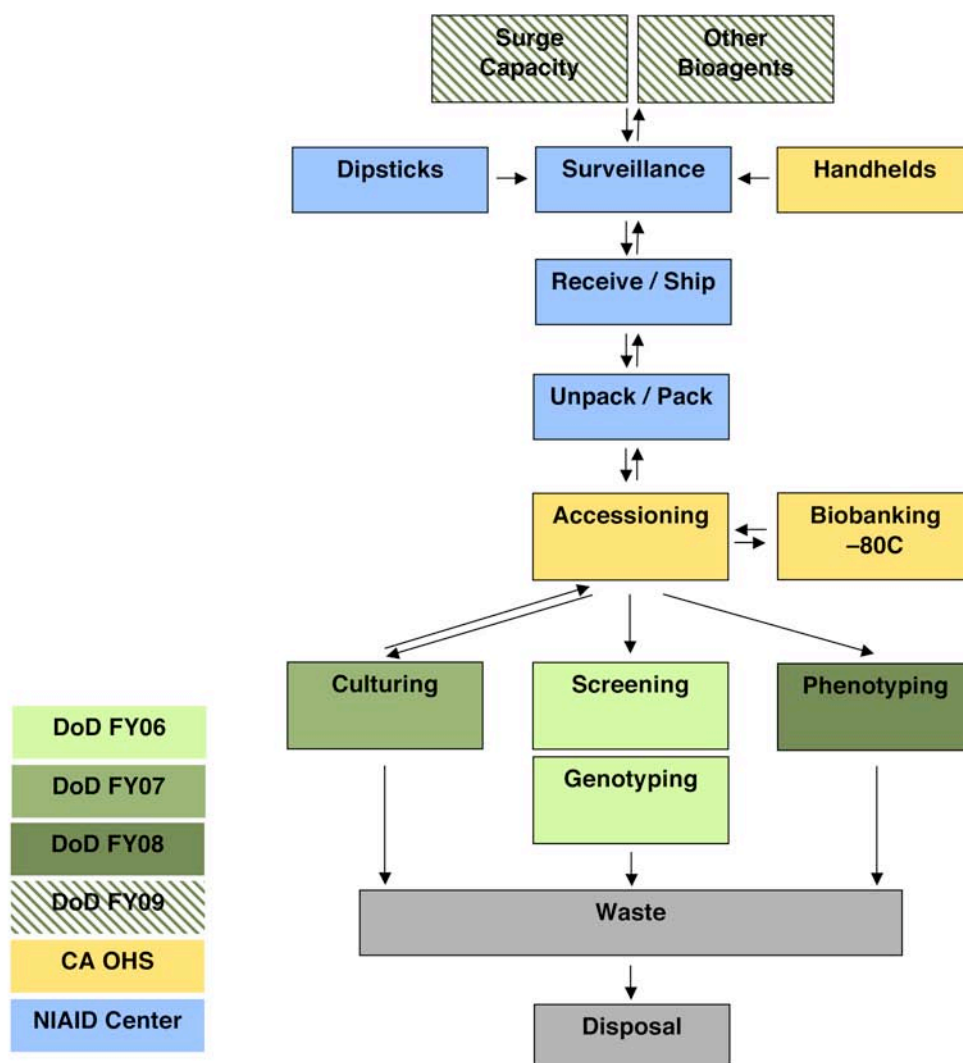
Figure 1. Automated laboratory systems shown in terms of functional relationships, funding sources and timing. DoD funding of our program supports core automated systems, methods, and containment space upgrades. NIAID funding of the UCLA-based Center for Rapid Influenza Surveillance and Research (CRISAR) supports national and international influenza surveillance (sample streams) and dipstick development. CA OHS funding of laboratory capacity supports additional automated systems, field use handhelds and containment space upgrades that enhance high-throughput capacity.

Progress and accomplishments on the five tasks in our FY 2006 (initial year) Statement of Work are as follows.

Task 1: LANL & UCLA. Assemble two automated systems that perform high-throughput tests. An automated bioagent screening system will be used to determine whether individual samples contain influenza viruses. It will also type and subtype samples for amplification primer selection prior to sequencing. An automated bioagent genotyping system will be used to sequence whole viral genomes and/or individual gene segments. A chosen vendor (Velocity11) will assemble the two systems to exact specifications. An established team at LANL will validate the systems and install them at UCLA.

There are five steps in designing and assembling automated systems for use at UCLA. The first is to define, develop and/or refine assay methods that will run on these systems. The second is to formulate software and hardware specifications for these systems. The third is to procure these systems from a laboratory automation vendor. The fourth is to validate these systems at LANL. The fifth is to install these systems at UCLA. For the automated bioagent genotyping systems, steps 1 – 3 have been completed and steps 4 – 5 will be accomplished in 2008 and the first quarter of 2009. For the automated bioagent screening system, steps 1 – 2 have been completed and steps 3 – 5 will also be accomplished in 2008 and the first quarter of 2009.

Define, Develop and/or Refine Assay Methods: Influenza Clean Up
Influenza viruses from animal and/or human surveillance consist of mixtures of respiratory secretions, plasma proteins, bacteria and viruses, cloacal and fecal matter, and environmental contaminants. The ability to detect (+/–), type (A vs. B), subtype (H and N) and/or sequence influenza viruses directly from surveillance samples depends on the ability to separate influenza viruses from these contaminants. We have therefore developed sensitive and specific methods to clean up influenza samples that are amenable to high-throughput laboratory processes. It utilizes magnetic beads that are coated with synthetic sialic acids. The milestones in such methods development are as follows.

• LANL has established a library of sialic acids attached to ethylene glycol spacers with 3, 5, 7, and 11 glycol repeats and attached them to non-specific background resistant coated MagSil magnetic beads in 0.1, 0.5, 1, 2, and 5% surface coverage (see Figure 2).

• The capturing assay with cultured influenza samples show viral captures efficiencies of up to 95%. Peg 3 at 1 % surface coverage appears to work best (see Figure 3). An assay testing the non-specific protein carryover using RNAse showed low residual RNAse activity retained on the beads.

• LANL will further optimize the beads, test their stability towards environmental degradation and extend the assay to complex sample matrices in the coming quarters.
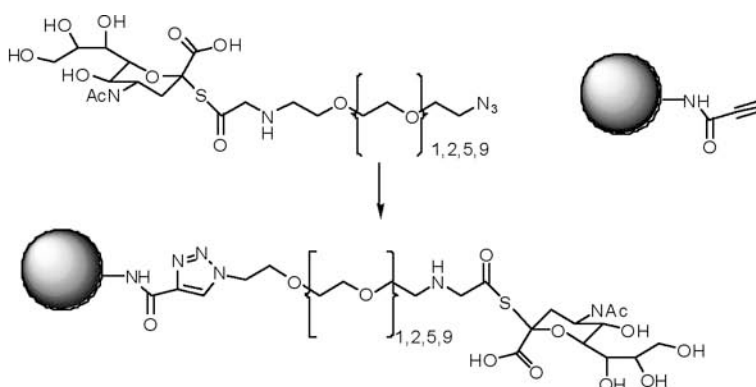
Figure 2. Molecular structure N-acetylneuraminic acid (sialic acid) derivatives.

Define, Develop and/or Refine Assay Methods: Influenza RNA Extraction
To quantify the efficiency of the above influenza clean up methods, LANL obtained 50 different cultured influenza viruses from surveillance work in collaboration with UCD. It performed cleanup up, RNA extraction and viral load quantification on these samples and delivered the samples to sequencing methods development team at LANL for subsequent analysis.

• A direct comparison of influenza clean up and capture by sialic acid versus clean up and capture by conventional immunologic methods demonstrated that carbohydrate ligands were 10-fold more effective than the conventional immunological magnetic beads methods.

• The newer sialic acid methods are now being optimized with the use of quantitative viral PCR and genome sequencing methods.

Figure 3. Quantitative influenza clean up and capture with sialic acid methods versus conventional immunologic methods. In each pull down experiment, 100,000 RNA copies of A/Sydney/5/97 were u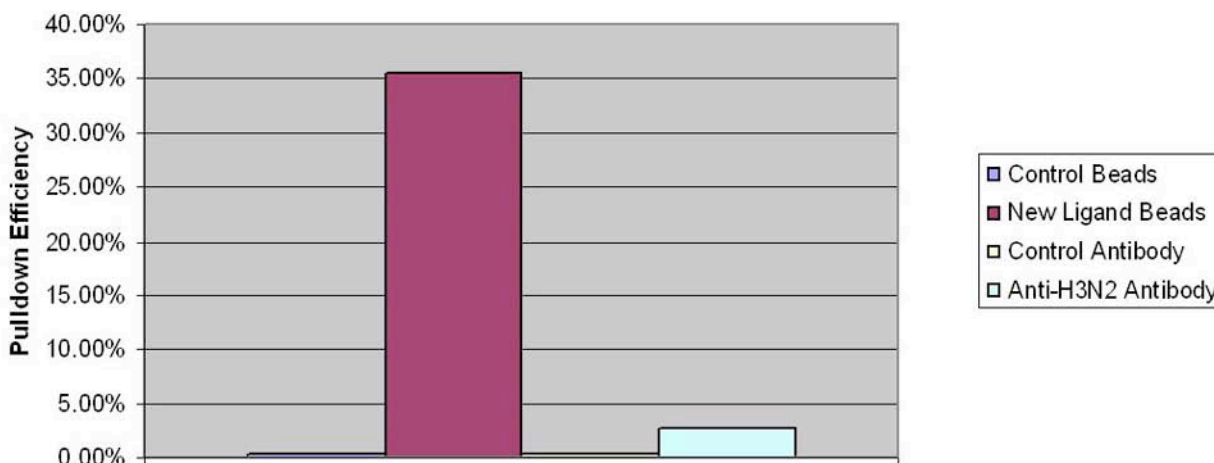sed. Sialic acid coated beads consisted of sialic acid with a PEG-3 spacer and at 1% coverage on the surface of magnetic silicon beads. Immunoglobulin coated beads consisted of high affinity anti-N3N2 on the surface of magnetic silicon beads. Control beads consisted of unmodified magnetic silicon beads. Results are the median of two independent virus pull down experiments. Background absorption (about 100 viruses per 100,000 input viruses) was subtracted from the data. For sialic acid and antibody controls, the pull down efficiencies are at background levels.

Define, Develop and/or Refine Assay Methods: Influenza Genome Sequencing
The influenza virus genome consists of eight RNA gene segments with a total length of ~13,600 bases. For influenza A, there are 16 hemagglutinin and 9 neuraminidase subtypes representing wide sequence variations. For influenza B, there is only one subtype with lesser yet significant sequence variations. The ability to whole genome sequence influenza viruses depends of having sufficient RNA copies and primer sets that can cover most if not all sequence variations. Since very little sequence information exists for may influenza subtypes, PCR primers will be updated and improved in an iterative fashion. With this process, a library of PCR primers will be assembled that permits sequencing of all RNA segments, including all hemagglutinin (H1–H16) and neuraminidase (N1–N9) subtypes. The refined methods developed by LANL for influenza screening and whole genome sequencing (genotyping) are shown in Figure 4 below.

Define, Develop and/or Refine Assay Methods: Primer Set Selection
The development of primer sets for influenza virus screening and whole genome sequencing is an iterative and open ended process. Over time, additional sequence information will lead to better primer set selections. The attached paper "Algorithms for Designing the Minimal Set of Multiplexed Degenerate Universal Tagged Primers for RNA Virus Detection" describes progress to date by LANL. Additional accomplishments include the following.

• Developed new primer sets for influenza samples from West Africa. They consist of degenerate TaqMan assay primers for MP, HA and NA gene segments at two different TMs (60C and 68C). They are anticipated to perform better than the existing primers.

• Implemented new computational algorithms to evaluate monoplex and multiplex primer set(s) coverage against known influenza strains. It uses sequence data from GenBank and LANL influenza databases, determines coverage of primer set against four different subtypes, and identifies strains that are not covered by current primer sets. Computational results are then tested against experimental results.
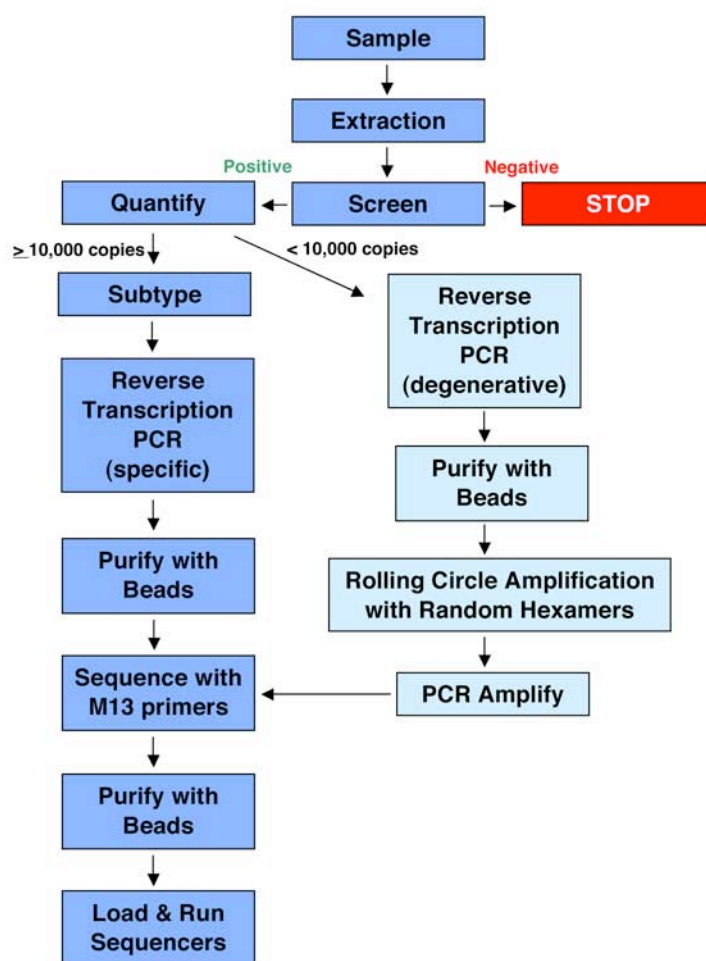
Figure 4. Screening and genotyping protocols for influenza viruses. **1)** Upon arrival, samples will be lysed with chaotropic reagents, RNA-captured with charged magnetic beads that undergo several washes, and released for RT-PCR screening reactions. Eluted RNA from these extractions will be primed with influenza matrix gene primers, converted into cDNA, PCR amplified, and detected with time-course fluorescent signal generation. **2)** For each sample, quantification of viral load will be determined by time-course fluorescence signal intensity versus cycling time (real time PCR) and viral copy number will be extrapolated by pre-calibrated viral RNA standard curves. **3)** Subtyping of HA and NA RNA genes will be carried out by Taqman PCR reactions and a library of subtype-specific amplification primer sets. **4)** As shown on the left side, RT-PCR will use specific primers linked to standard M13 sequencing primers. This will produce overlapping 200 – 300 base pair double-stranded cDNA fragments for high-throughput sequencing by a single standard sequencing primer for each direction (forward and reverse). The cDNA generation process will be carried out in low volumes (5 μl) and each sequenced sample will be arrayed within a single 96-well PCR plate. **5)** As shown on the right

side, a secondary amplification method will be used, if RNA copy numbers are less than 10,000. Degenerative influenza primers will be utilized to amplify all 8 gene segments and followed by magnetic bead cleanup and wash step. Rolling circle amplification with random hexamers will be carried out to generate sufficient DNA for specific adaptor PCR and sequencing as descried above. **6)** For greater than 10,000 or less than 10,000 copy-based methods, high-throughput sequencing with standard M13 primers will be carried out with Big-Dye chemistry on ABI 3730xl capillary DNA sequencers.

Software/Hardware Specifications and System Procurement: Genotyping System
LANL completed the software and hardware specifications for the automated bioagent genotyping system in January 2008. The UCLA Request for Proposals (RFP) for genotyping system was issued on March 6, 2008 and will close on April 11, 2008. According to the RFP's schedule, the contract award will be in May 2008 and the delivery date to LANL will be September 2008 (see Appendices). Following delivery, the genotyping system will be validated by LANL and will be moved to UCLA by the vendor in March 2009, when he BSL3e facility is scheduled for opening and operation.

Software/Hardware Specifications and System Procurement: Screening System
LANL completed the software and initial hardware specifications for the automated bioagent screening system in March 2008. The next step in the procurement process will be to issue the UCLA Request for Proposals (RFP) in the second quarter of 2008.

Task 2: LANL. Build an operating system that runs and manages networking of multiple high-throughput laboratories. The operating system will allow access by way of the Internet, enable flexible and programmable testing procedures, schedule and control numerous tests, and deposit results into an Internet-enabled database for analysis, geospatial mapping and real-time display. An established team at LANL will accomplish this task.

With FY 2006 (initial year) DoD support, we began work on an operating system that enables the above capabilities. The operating system's architecture is based on three United States Patents (Layne and Beugelsdijk 1998, 1999 and 1999), takes advantage of the Laboratory Equipment Control Interface Specification (American Society for Testing and Materials 1999) and utilizes commercial hardware and software packages (Layne and Beugelsdijk 1998).

The overall operating system is implemented by an n-Tier Information Technology (IT) architecture that enables computing platform-independence, vendor-neutral data storage and retrieval, plug-and-play compatibility and scalability. To achieve these attributes, we logically partition the operating system into five layers (Physical, Data, Business Logic, Presentation, and Application). We also utilize open IT standards, such as web services for interoperability across heterogeneous computing platforms, eXtensible Markup Language (XML) for data exchange, and Open Database Connectivity (ODBC). Table 1 outlines the purpose and function of these five layers. Progress on the various aspects of this operating system are summarized below.

Table 1. n-Tier IT Architecture

Layers	Description
Application	The Application layer hosts internal and external, business-to-business and/or user applications.
Data	The Data layer manages all data generated by or submitted to the laboratory. Databases, flat files and backup tapes are examples of items residing at this level.
Business Logic	The Business Logic layer defines how the laboratory operates internally and how to conduct business with it (e.g., how to submit samples, process samples, manage report and conduct Enterprise Resource Planning (ERP).
Presentation	The Presentation layer defines how information exchanged and presented to applications from the Application Layer (e.g., via web forms, ASP.NET server pages and XML/SOAP).
Physical	The Physical layer collectively refers to all laboratory facilities, automated (and non-automated) laboratory systems and functional subsystems.

<u>IT Hardware</u>
At present, two DELL PowerEdge 2950 machines function as database servers and one PowerEdge 1950 machines functions as the web-enabled server (Figure 5). Currently, these two database machines are situated at LANL but, during the second quarter of 2008, one of these machine will be move and connected by a fiber optic network (with 1 – 10 gigabit per second bandwidth) that is available to these two institutions (Figure 6). Table 2 summarizes the current configuration of this IT hardware and software and planned improvements in FY 2007 (option year 1) and FY 2008 (option year 2).

• Note: In 2007, major hardware and software procurements were funded by our NIAID Center contract and greatly enhance the value of our DoD supported program.

<u>Laboratory Operating System (LOS)</u>
This includes custom-developed, internet-enabled "operating system" services for the high-throughput laboratory, such as real-time data subscriptions and visualizations, and tools for remote laboratory node administration and federation. These LOS services will be transparent to the user and communicate directly with the Laboratory Information Management System (LIMS) to enable access to all LIMS-managed laboratory data. Any LOS service-related information that is not directly managed by the LIMS will be stored in a highly scalable, relational, mirrored Microsoft SQL Server-based database. Table 2 summarizes the current operational status of the LOS and planned improvements in FY 2007 (option year 1) and FY 2008 (option year 2).

<u>Laboratory Information Management System (LIMS)</u>
This provides distributed, computing platform-independent, data acquisition and lifecycle management services for the high-throughput laboratory. It will enable multiple tasks such as: linked sample-result data tracking, laboratory QA/QC management, device and human resource supervision, instrument performance monitoring and calibration, billing records and supply chain management (Figure 7). The commercial software product that we have selected for these tasks is called *StarLIMS*. It is a 100% web-based LIMS solution that enables access real-time laboratory information via a simple web browser-based user interface. *StarLIMS* stores data in a highly scalable, relational and mirrored Microsoft SQL Server-based database. To simplify logistics, system maintenance and reduce IT costs, the SQL server database used by *StarLIMS* will be the same as the one being used by the LOS.

• With FY 2006 (initial year) funds, the *StarLIMS* software package was procured in March 2008 by a UCLA RFP (see Table 2).

Table 2. Summary of Three-Year Implementation Plan

System Components	Year	Activities
IT Hardware	1	• Procure DELL PowerEdge 2950 (data) and PowerEdge 1950 (web) server hardware and software.* • Implement DELL server hardware and software in a fault-tolerant manner using server clustering in Windows Server and EMC Replistore. Achieve reliable, quasi real-time server and data mirroring over a Wide Area Network (WAN). • Transfer UCLA PowerEdge 2950 and PowerEdge 1950 servers to UCLA during Quarter 2, 2008. • Test wide-area server mirroring capabilities and system recovery protocols by simulating hardware and software failures on the UCLA and LANL servers. • Train UCLA staff on server operation and maintenance.
	2	• Expand PowerEdge 2950 RAID storage from 1TB to 4TB of active, online storage.* • Add 2nd Intel 1.6GHz Quad Core CPU to PowerEdge 1950 web server.*
	3	• Add automated EMC long-term data storage and retrieval system.
Laboratory Operating System (LOS)	1	• Implement basic web services interface for remote subscription to laboratory and result status updates.
	2	• Integrate a graphical, web-based, real-time situational awareness service using Google Earth. The service will interactively visualize incoming sample streams and also visualize the laboratory's current operational status (number of samples in queue, average turnaround, available capacity, etc.).
	3	• Implement advanced, web-based, networking services for on-the-fly laboratory network node discovery, laboratory node federation, and sample load balancing (to assist in distributed decision making during a pandemic). • Implement user capabilities (e.g., provide data links and/or custom web service interfaces) that facilitate integration and use of 3rd party software tools (e.g., scientific visualization programs, statistics packages, simulations, multi-dimensional data mining tools, etc.)
Laboratory Information Management System (LIMS)	1	• Procure *StarLIMS* package.† • Implement web-based sample submission forms that enable automated, standard laboratory workflows for sample submission, QA/QC, sample storage/retrieval, off-site shipping, sequencing and reporting. • Implement meta-data models for field surveillance and genotyping and configure database schemas. • Implement data import/export web-services interface for accessioning, screening and genotyping systems. • Train UCLA staff to use LIMS.
	2	• Implement meta-data model for culturing system and expand LIMS database schemas. • Implement forms and automated work flows in *StarLIMS* to accommodate culturing system. • Implement data import/export web-services interface for biobanking and culturing systems.
	3	• Implement meta-data models for phenotyping and configure database schemas. • Implement forms and automated work flows in *StarLIMS* to accommodate phenotyping system. • Implement data import/export web-services interface for phenotyping system.

*Procurements funded by the NIAID Center.
†Procurements funded by the DoD program.
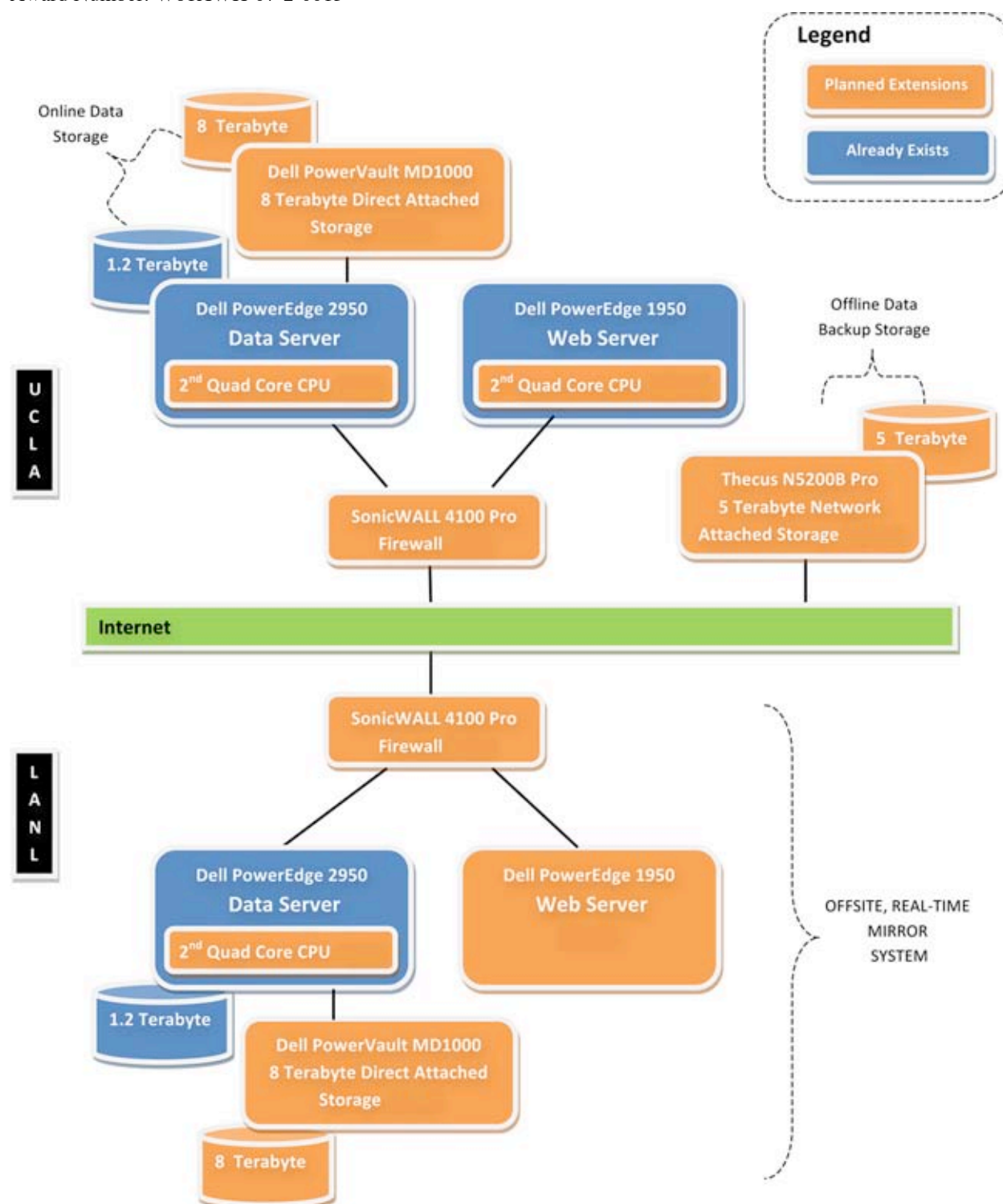
Figure 5. High-throughput laboratory's IT hardware and software components. Blue boxes depict components already purchased and in operation with NIAID support. Yellow boxes depict components to be purchased to achieve full data management capabilities with anticipated NIAID support. **Note:** All hardware and software components shown in Figures 5 and 6 were procured with NIAID-based funds with the exception of the Laboratory Information Management System (LIMS) from *StarLIMS*, which was procured with congressionally directed DOD-based funds. These NIH/NIAID funds greatly complement and enhance our congressionally-directed DoD funded program.
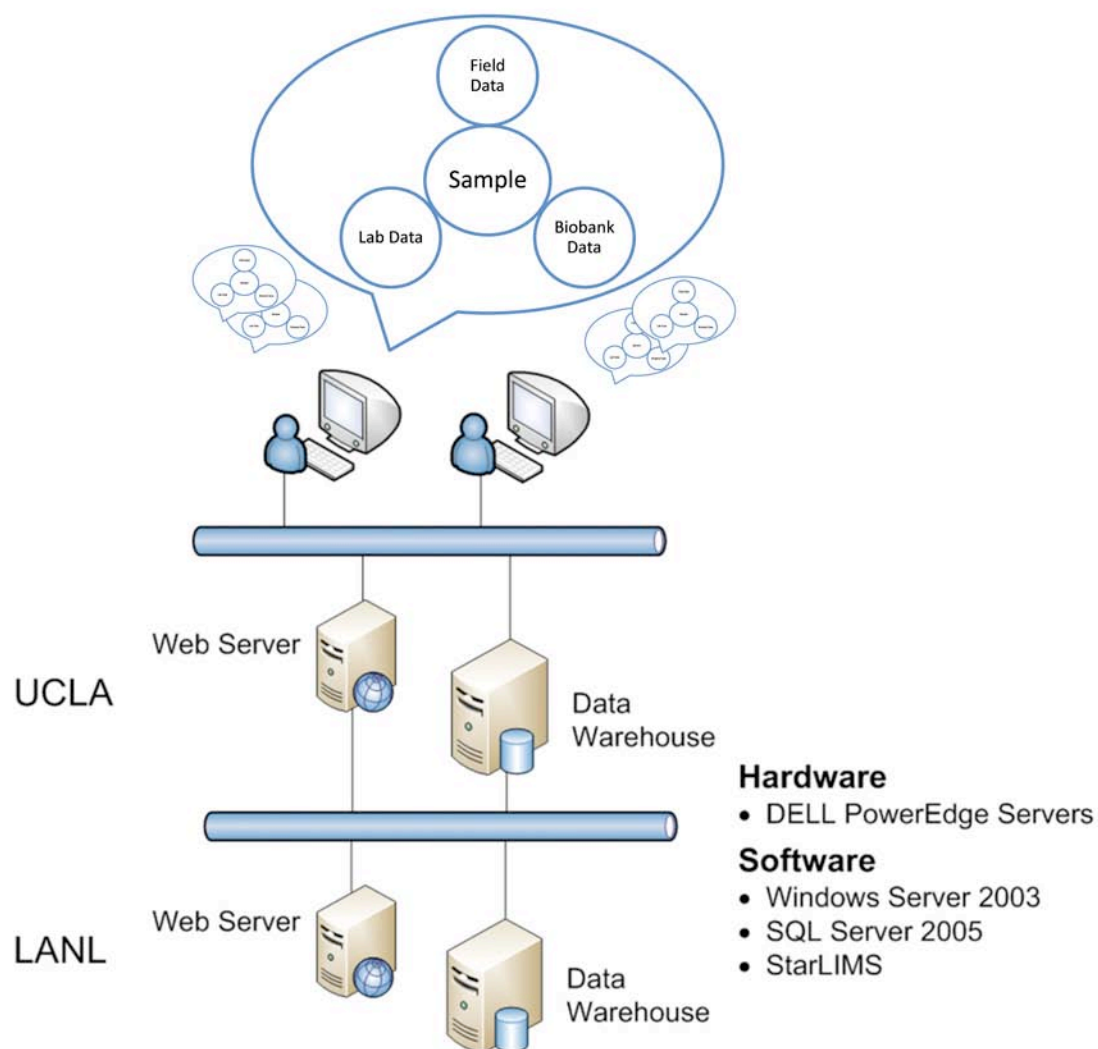
Figure 6. High-throughput laboratory's IT infrastructure provides two mirrored and fault-tolerant server systems that will soon be connected by the ultra high-speed (up to 10 gigabyte per second) fiber optic network that links UCLA with LANL. Each sample will be associated with field-based surveillance data, laboratory-based genotypic and/or phenotypic data, and laboratory-based sample biobanking data. Web servers provide convenient user interfaces to data warehouse servers.
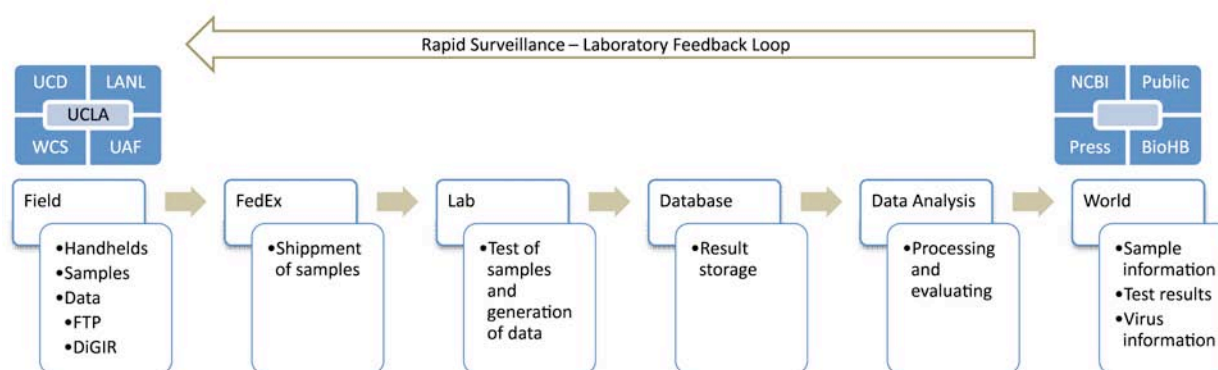
Figure 7. NIAID Center participants and data flows for the High Speed, High Volume Laboratory Network for Infectious Diseases.

Task 3: UCLA. Upgrade ~2,000 sq. ft. of floor space to BSL3+ and/or BSL3-Ag specifications for housing high-throughput automated systems. Containment space will be situated in the California NanoSystems Institute (CNSI) which has assigned new space for the proposed program. The space will facilitate the flow of numerous samples, reagents, supplies, waste and information through it. Highly pathologic avian influenza (HPAI) is a select agent that must be handled in biosafety containment with chain of custody. Initial focus on HPAI will thus expedite preparedness for other select agents. UCLA will accomplish this task with a chosen architecture, engineering and planning vendor that specializes in laboratory work.

Since submitting our FY 2006 (initial year) proposal, The *Biosafety in Microbiological and Biomedical Laboratories, 5th edition* was released, which specifies that HPAI must be handled in BSL3-enhanced (BSL3e) containment space (U.S. Department of Health and Human Services 2007). The upgrade of floor space is therefore complying with the newer BSL3e specification rather than the older BSL3+ and/or BSL3-Ag specifications.

Containment space for the UCLA High Speed, High Volume Laboratory Network for Infectious Diseases must be capable of receiving and testing all influenza viruses, including those designated as Highly Pathologic Avian Influenza (HPAI) strains. Federal regulations also require that HPAI be handled as a Select Agent in accordance with 7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73.

UCLA has assigned 6,300 sq ft of floor space in its new California NanoSciences Institute (CNSI) building to house the high-throughput program. As required, this space is being upgraded to BSL3e containment specifications. In November 2006, UCLA selected the architectural, engineering and planning firm CUH2A to design the high-throughput facility. CUH2A specializes in laboratory work and has designed many BSL3/BSL4 facilities, including the new United States Army Medical Research Institute for Infectious Diseases (USAMRIID), NIH Center for Biodefense and Emerging Infectious Diseases, and CDC Emerging Infectious Disease laboratories (see htttp://www.CUH2A.com). In November 2007, 100% design plans for the high-throughput facility were completed and facilities upgrade work was begun (see Figure 8). The upgrade and commissioning phases will last through March 2009 and transfer of the first three automated systems (accessioning, genotyping, screening) from LANL to UCLA is also scheduled for March 2009. Installation of the automated biobanking system is scheduled for March. High-throughput BSL3e operations will begin soon after the first three automated systems are installed at UCLA.
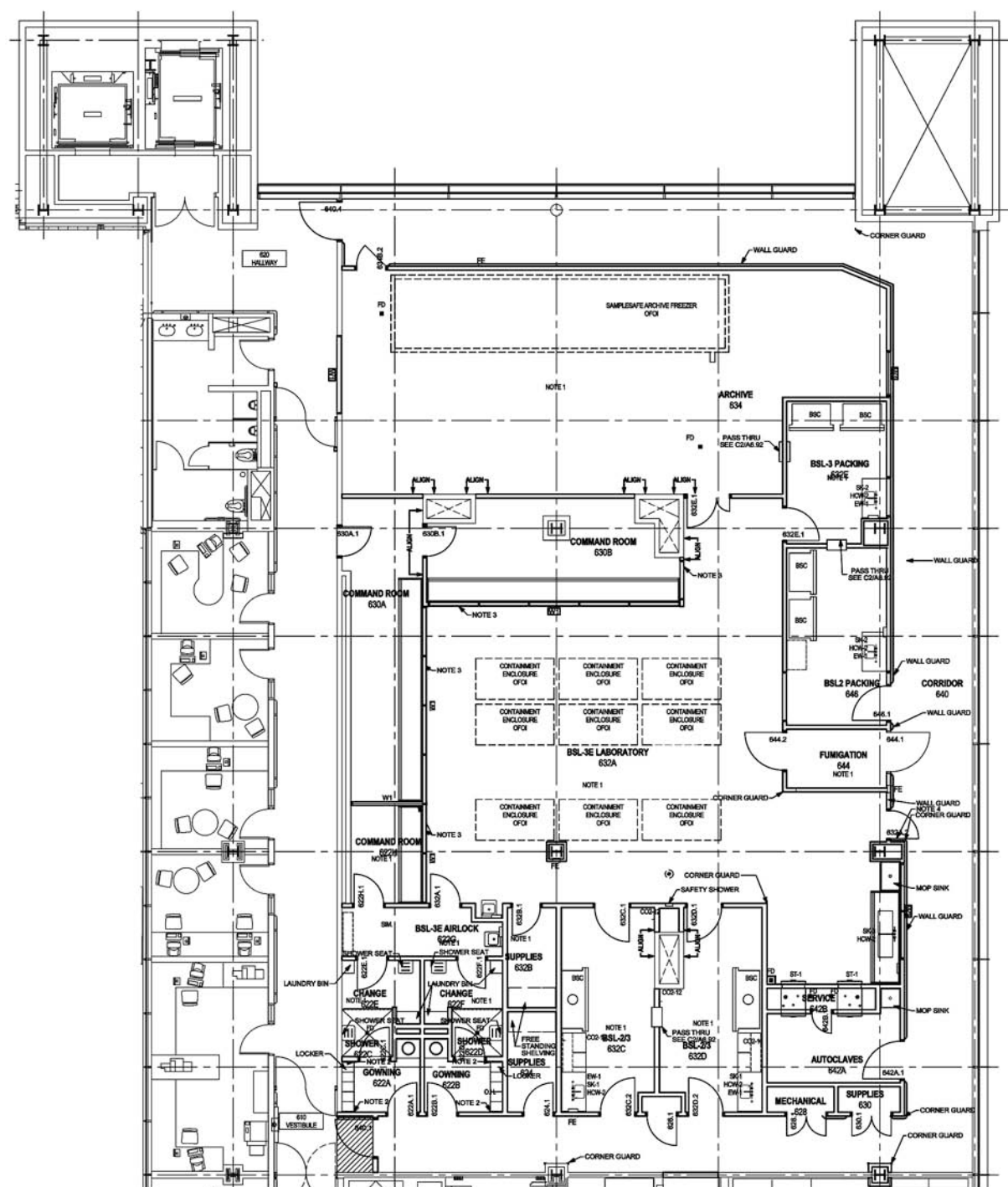
Figure 8. The BSL3e facility will be situated on the top floor of the new UCLA CNSI building. The central containment area is configured to house automated laboratory systems in individual enclosures. The drawing depicts 100% design plans by CUH2A.

Task 4: LANL & UCLA. Formulate quality management program and protocols, and establish team that operates the high-throughput laboratory. An established team at LANL will scale up procedures for screening and genotyping influenza viruses. In parallel, LANL will develop a quality control program that ensures reliability, reproducibility and error reduction. A new team at UCLA will manage and operate biocontainment facilities and automated systems. To expedite this task, the LANL and UCLA teams will work together closely.

With FY06 (initial year) DoD support, we initiated work on a formalized quality assurance/quality control (QA/QC) program and on sensitive and specific methods for genotyping and screening influenza viruses. Our progress to date is summarized below.

Quality Management
The high-throughput laboratory network initiated the development a formalized quality assurance/quality control (QA/QC) program that covers processes from field sample collection to data quality and surety in order to eliminate or minimize the introduction of errors. The quality management system will describe the QA/QC programs and describe approaches to ensure that program requirements are met. The quality management system will describe procedures for conducting audits in the following areas: 1) equipment maintenance and repair; 2) training records and adherence of staff to required training schedules; 3) data management; 4) record keeping and records management; and 5) adherence to standard operating procedures. While relying on the LANL and UCLA team to develop best practices, the quality program will capture and codify these into formal processes and procedures for current and future staff and affiliates.

Genotyping
The automated genotyping system will sequence all eight RNA segments or any individual segment from influenza viruses (Table 3). The various steps performed are summarized in Figure 4. Influenza viruses are often propagated in cell cultures or embryonated eggs to obtain enough viral RNA for sequencing (Xu 2002, Barr 2005, Holmes 2005). Newer methods that avoid this growth step, however, will facilitate direct high-throughput analysis of native samples from surveillance. This will include active samples preserved by cold chain as well as inactive samples preserved by chemical inactivation (Krafft 2005, Ilyushina 2005, Runstadler 2007). Since very little sequence information exists for may influenza subtypes, PCR primers will be updated and improved in an iterative fashion (see paper by Song et al in Appendices). With this process, a library of PCR primers will be assembled that permits sequencing of all RNA segments, including all hemagglutinin (H1–H16) and neuraminidase (N1–N9) subtypes.

Table 3. The Genes and Proteins of Influenza A

RNA Segment	Size in Bases	Name and Abbreviation	Major Function	Copies per Particle*
1	2,341	Polymerase, PB1	RNA transcription/replication	30–60
2	2,341	Polymerase, PB2	RNA transcription/replication	30–60
3	2,233	Polymerase, PA	RNA transcription/replication	30–60
4	1,778	Hemagglutinin, HA	Viral attachment/penetration	500
5	1,565	Nucleoprotein, NP	Viral RNA synthesis/stability	1,000
6	1,413	Neuraminidase, NA	Viral release	100
7	1,027	Matrix, M1	Assembly/regulation	3,000
		Channel, M2	Viral entry/pH regulation	20–60
8	890	Nonstructural, NS1	Interferon antagonist	–
		Nuclear export, NEP	Viral assembly	?

Influenza A contains eight different RNA segments (13,588 bases) that code for ten proteins. Six segments code for one protein whereas two segments code for two proteins. Influenza B has a similar genome. *Approximate numbers.

<u>Screening</u>.
The automated screening system will detect, quantify, type (A vs. B) and subtype (H1–H16 and N1–N9) influenza viruses (Lau 2004, Moore 2004, Ng 2005, Payungporn 2006). It will work with samples taken from various physiologic sources including cloacal, upper respiratory and blood specimens (Runstadler 2007). The various steps performed are summarized in Figure 4. Since very little sequence information exists for may influenza subtypes, RT-PCR probes will be updated and improved in an iterative fashion (see paper by Song et al in Appendices). Screening assays will be optimized to achieve absolute detection limits (+/–) that approach 100 RNA copies. Positive and/or subtyped samples will be passed to the automated genotyping system.

Task 5: UCLA & LANL. Conduct large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses. UCLA and LANL will initiate a viral "pathomics" effort to understand how influenza strains are evolving and which ones pose threats. The effort will allow scientists and health officials to judge threats posed by particular subtypes and strains.

Pandemic influenza constitutes one of the foremost "grand challenges" of our time (Layne 2001, Holmes 2005). Critically important gaps, however, remain in our basic knowledge and understanding of influenza viruses — even though they have been studied for over 70 years (Francis 1940). A new and practical approach to influenza surveillance, research and response is therefore needed (Layne 2006).

The ability of influenza viruses to reproduce, mutate and select for new pandemic strains is well recognized. It is therefore only a matter of *when* and not *if* a pandemic will emerge and how severe it will be (Homeland Security Council 2006). It has also become clear that viral RNA mutations governing key properties related to virulence, transmissibility and host range are polygenic in origin, rather than caused by simple point mutations (drifts) or gene segment substitutions (recombination and shifts). Because of this, the combinatorial space occupied by influenza's genome (8 RNA segments with total length of ~13,600 bases) is enormous (Figure #).

• We hypothesize that influenza viruses worldwide constitute a complex system that requires large-scale, data-driven efforts for greater understanding of their key properties. Computer intensive analyses of which will identify various polygenic mutations (molecular order and patterns) associated with virulence, transmissibility and host range.

• We hypothesize further that three domains of data are required for seeking such greater understanding. *Epidemiologic* pertaining to dates, locations, hosts, outcomes, histories and exposures. *Genotypic* pertaining to the exact sequence of nucleotides in all eight viral RNA segments. *Phenotypic* pertaining to immunologic pedigrees and antiviral drug sensitivities of viral strains for example.

<u>Progress to Date on Data-Driven and Hypotheses-Based Research</u>
LANL has received 50 cultured influenza viruses from animal surveillance work conducted from the University of California Davis (UCD). Using methods that will be implemented on automated bioagent systems, LANL performed RNA extraction, reverse transcription and polymerase chain reaction amplification (RT-PCR), and sequencing with an avian "universal" primer set that were obtained from the Venter Institute. This primer set is comprised of 192 primer pairs which fit into two 96-well plates. The primer set was used to amplify all eight influenza RNA genome segments in overlapping regions. As shown in Figure 4, a universal sequencing primer (M13) was then used to sequence the amplified DNA products with an Applied Biosystems 3730xl DNA analyzer.
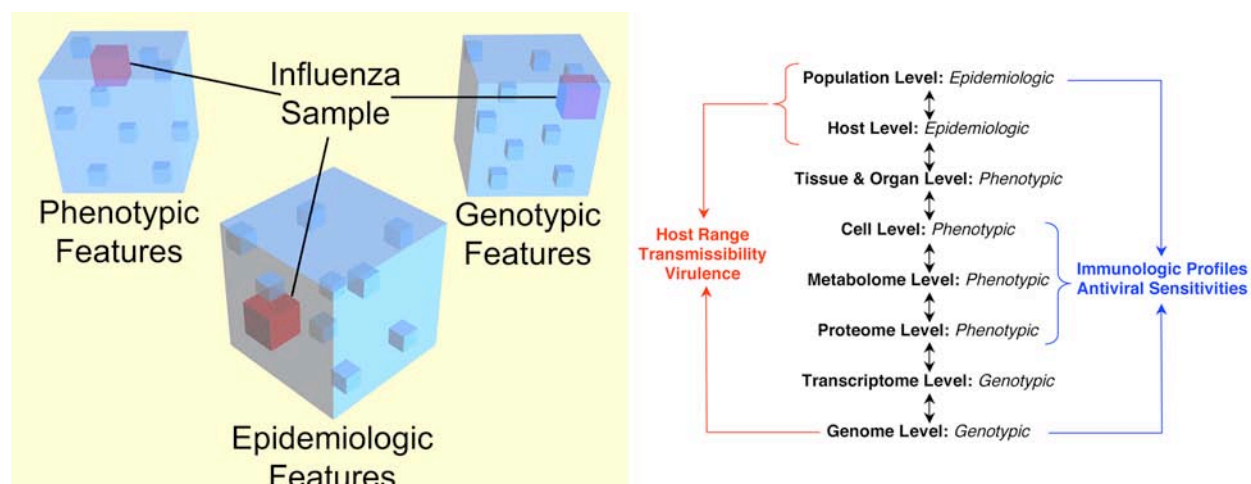
Figure 9. When fully operational, the high-throughput laboratory network would give rise to these three domains of associated data (left). These data will enable much needed epidemiologic, genotypic and phenotypic associations (right).

The Phred/Phrap/Consed software package (www.phrap.com) was used for sequence assembly and quality assessment. After assembling the sequences manual, editing (joining, tearing contigs, etc.) was performed on the 50 individual databases. For each starting sample, the eight segments were carefully examined for intact ORF and trimmed on both ends. After the first round of sequencing, 14 samples showed complete coverage. If the coverage was less than 70%, we repeated the sequencing with all 192 primer pairs. If the coverage was greater than 85%, individual PCR pairs were selected for sequencing again (repeats) in the following manner. A database was created that included all the sequencing data from the 50 samples. Local BLAST search was performed against our database using the incomplete segment as a query. This comparative procedure enabled identification of complete segments that showed the highest similarity to incomplete ones and, by comparing them, it also enabled determination of which PCR reactions to repeat. Altogether, 213 PCR reactions were performed again (repeated) to cover the gaps in 26 flu samples. In order to add the new (repeated) PCR fragments to the existing databases, the "add new reads" option was used in Consed to avoid reassembling all the sequences. With such methods, an additional 12 samples showed complete coverage after the second round of sequencing.

For the 50 cultured influenza viruses from animal surveillance, the following results were obtained: 26 samples yielded 100% whole genome sequences; 10 samples yielded 95.0 – 99.7 whole genome sequences; 6 samples yielded 91.5 – 99.7 whole genome sequences; 4 samples yielded mixed results; and 4 samples contained insufficient viral RNA. This work with authentic avian surveillance samples serves as a basis for improving automated bioagent methods and quality controls.

LANL is currently placing these new whole genome sequences into trees that are composed of 330 representative whole influenza genomes that cover host range diversity (human, avian, swine, etc.), time points of collection, surface protein immunotypes (H1–16, N1–9), and geographic diversity. This analysis includes separate trees for each of the eight gene segments in both nucleic acid and amino acid space (see Figure 10).
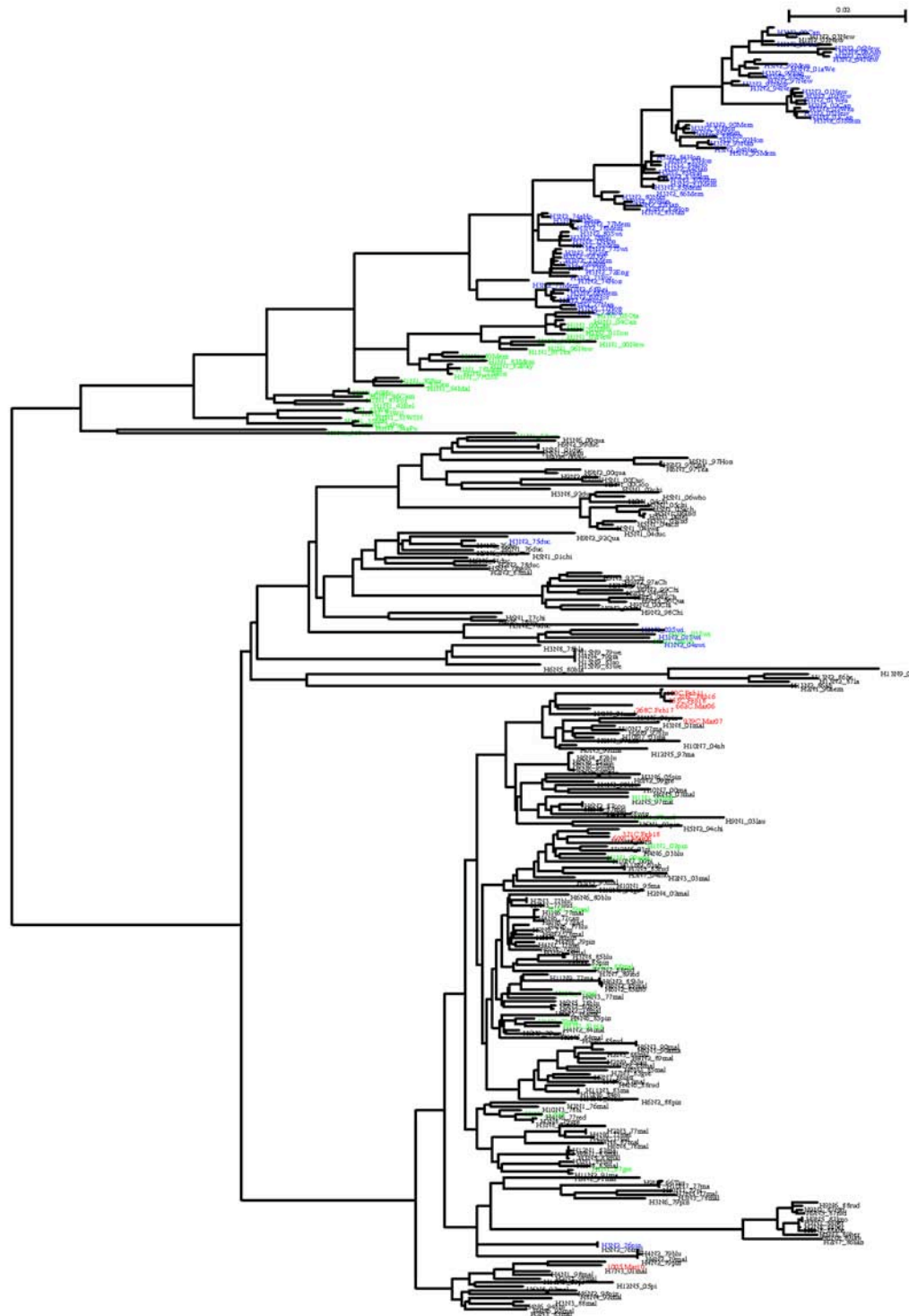
Figure 10. The figure shoes an influenza genome analysis tree. Nine of the new influenza genomes (shown in red) placed in context of known phylogenetic diversity of the nucleoprotein (NP, segment 5). The three major divisions in this tree represent human samples (top) and avian samples from the eastern and western hemispheres (bottom). As expected, the new sequences all cluster with western hemisphere viruses. Some new sequences match with various existing clusters and others are fairly distant from known sequences. Two of the main serotypes afflicting humans are colored (H1N1 in green and H3N2 in blue).

In addition, LANL is beginning the search for re-assortant viruses in these new whole genome sequences by building and examining trees composed of concatenated amino acid tree from all eight gene segments. This work serves as the foundation for additional computationally-intensive analyses where ~3,000 published whole genome sequences (in GenBank) will be added to our 50 new (and soon more) whole genome sequences. These whole genomes will then be related to their original host (human, avian, swine, etc.) and the resulting ~3,050 concatenated whole genomes (in nucleic acid and amino acid space) will than be comprehensively searched for all polymorphisms associated with host range. LANL has and will apply institutional computational resources appropriate to this CPU-intensive task.

Scientific Advisory Board (SAB). On April 25, 2007, the program's first SAB meeting was convened at UCLA. The board was expanded from the initial four members to ten members from a variety of relevant disciplines. SAB members present included: Nancy J. Cox, Margaret Hamburg, Virginia Hinshaw, King Holmes, Colonel George W. Korch Jr., Elizabeth Wager, and Laurie Zoloth. SAB members absent included: Peter B. Jahrling, David E. Swayne, Jeffrey K. Taubenberger. Those present from LANL included: Tony J. Beugelsdijk (Co-PI) and Gary Resnick. Those present from UCLA included: Dean Linda Rosenstock, Assistant Dean Anderson, Assistant Dean Roshan Bastani, Hilary Godwin, Assistant Dean Kathleen Kiser, Scott P. Layne (PI), Anne Rimoin, Marisa Cortes Weber, Nathan Wolfe, Rebecca Wolfe. Following a scientific and technical introduction to the program, valuable feedback and advice was elicited from SAB members. Over the initial year, we have been in touch with SAB members for directed feedback and advice as needed. The second SAB meeting is planned for the fall of 2008.

KEY RESEARCH ACCOMPLISHMENTS

With FY 2006 (Initial Year) DoD Funds

• Produced design specifications for a genotyping system. UCLA has issued an RFP for this system and will proceeded with its purchase in April 2009. The genotyping system is scheduled for delivery to LANL in September 2008 for validation and operation. It will be moved to UCLA in March 2009.

• Began development of design specifications for a screening system. These efforts will result in the purchase and delivery of another system by December 2008.

• Began development of an operating system that will manage and control high-throughput laboratory systems.

• Evaluated Laboratory Information Management Systems (LIMS) from multiple vendors and UCLA purchased the best suited software system from *StarLIMS*. This LIMS will manage all data generated by high-throughput laboratory systems.

• Hired the architectural firm CUH2A to design a new BSL3-enhanced laboratory facility and 100% design plans have been completed. Requested and evaluated bids from qualified vendors and selected a winning vendor in April 2008. The 6,300 square foot facility is scheduled for opening at UCLA in March 2009.

• Developed significantly improved laboratory methods for the whole genome sequencing of influenza viruses. Began initial efforts to conduct large-scale, data-driven research on influenza viruses.

With Complimentary California Office of Homeland Security Funds

• Purchased automated accessioning system from Tecan that accepts, divides and reformats infectious disease samples for subsequent testing and analysis. The accessioning system was delivered to Los Alamos National Laboratory (LANL) in December 2007 for validation and will be moved to UCLA in March 2009.

• Purchased automated biobanking system from REMP that stores and manages up to 800,000 samples at –80C. The system is scheduled for delivery to UCLA in March-April 2009.

• Hired the architectural firm CUH2A to design a new BSL3-enhanced laboratory facility and 100% design plans have been completed. Requested and evaluated bids from qualified vendors and selected a winning vendor in April 2008. The 6,300 square foot facility is scheduled for opening at UCLA in March 2009.

• Purchased 150 field use handheld devices from LANL that enable paperless surveillance and data collection efforts. Use of these handhelds by surveillance teams in the field will begin April 2008.

• Initiated a national search for a highly qualified director/manager of operations for the BSL3-enhanced laboratory facility. At present, our search continues.

With Complimentary NIAID Center Funds

• Established the Center for Rapid Influenza Surveillance and Research (CRISAR), which includes participation by the University of California Davis (UCD), University of Alaska Fairbanks (UAF), Wildlife Conservation Society (WCS) and LANL. The UCLA-based Center hired new staff, including Administrative and Information Technology (IT) managers, and began research operations.

• Conducted animal surveillance in California, Alaska, Japan, Russia, Mongolia, and Cambodia. These national and international efforts resulted in the collection and analysis of over 50,000 samples by subcontracting institutions. When the UCLA BSL3-enhanced facility opens in 2009, its high-throughput laboratory systems will test and analyze incoming samples.

• Sequenced 50 whole influenza genomes from surveillance samples collected in 2007. It will sequence another 50 whole genomes within the next few months. Efforts are now underway to analyze these new genome sequences.

• Began development of an "influenza dipstick" with cartridges for sample cleanup, reverse transcription and isothermal PCR-based amplification, and lateral flow detection by visual

readout. This effort aims for a detection sensitivity of 100 influenza particles, readout within 30 minutes and cost of $10 – $20 per dipstick.
• Purchased major hardware and software components for two mirrored and fault-tolerant data servers. These systems will soon be connected to the ultra high-speed fiber optic network that links UCLA with LANL and all other Internet-based networks worldwide. This setup will enable an advanced data generation, data management and information technology (IT) infrastructure for rapid and near real-time influenza surveillance, laboratory testing, result analysis and information sharing.
• Received second year funding for continued national and international surveillance and analysis of influenza viruses.
• Received supplemental support from the U.S. Department of Health and Human Services to begin groundbreaking animal surveillance activities in west Africa and human-animal interface studies in the same region.

REPORTABLE OUTCOMES

Manuscripts

Layne SP. Human Influenza Surveillance: the Demand to Expand. Emerging Infectious Diseases 12, 562 – 568 (2006).

Song J, Gans J, Wolinsky M, Li P-E, Curd C, Detter D, Cai H. Algorithms for Designing the Minimal Set of Multiplexed Degenerate Universal Tagged Primers for RNA Virus Detection. (Submitted to BIOCOMP'08 Conference).

Abstracts / Presentations

Layne SP. Invited Presentation: High Speed, High Volume Laboratory Network for Infectious Diseases. Southern California American Society for Microbiology Spring Symposium. Long Beach, California (March 21, 2007).

Layne SP. Invited Presentation: New High-Throughput Laboratory for Molecular Surveillance of Influenza. Wadsworth Center, New York State Health Laboratory. Albany, New York (April 30, 2007).

Layne SP. Invited Presentation: New High-Throughput Laboratory for Molecular Surveillance of Influenza. 10th Annual Conference on Vaccine Research. Baltimore, Maryland (April 30 – May 2, 2007).

Layne SP. Invited Presentation: Center for Rapid Influenza Surveillance and Research (CRISAR). 1st Annual NIAID Centers of Excellence for Influenza Research and Surveillance Network Meeting. Bethesda, Maryland (May 7 – 8, 2007).

Layne SP. High-Throughput Global Lab Against Influenza. Pandemic Influenza Symposium: California Association of Public Health Laboratory Directors. Long Beach, California (October 17 – 19, 2007).

Layne SP. Invited Presentation: High Speed, High Volume Laboratory Network for Infectious Diseases. Biological Threat Non-Proliferation Conference. Santa Fe, New Mexico (December 4 – 6, 2007).

Layne SP. Invited Presentation: High Speed, High Volume Laboratory Network for Infectious Diseases. Canada-California Infectious Diseases Collaboration Workshop. Los Angeles, California (March 2, 2008).

Informatics / Databases

Accession numbers for whole influenza genome sequences to be deposited with BioHealthNet (http://www.biohealthbase.org) and GenBank (http://www.ncbi.nlm.nih.gov/Genbank) are pending.

Funding Applied for Based on Work Supported by this Award

Principal Investigator: Scott P. Layne
Co-Investigator/Collaborators: Tony J. Beugelsdijk (Co-PI, LANL)
Title: UCLA High Speed, High Volume Laboratory Network for Infectious Diseases
Funding Mechanism/Agency: Congressional Appropriation through Department of Defense, Defense Threat Reduction Agency
Costs: $6,000,000 DoD FY 2007 Line Item
Duration: 1 year
Brief Synopsis: Second year of funding for project that aims to develop a new high speed, high volume (high-throughput) laboratory capability that will be linked in a network and operated by several premier institutions. The automated, networked capability will make us stronger against natural diseases and bioterrorist attacks.

Principal Investigator: Thomas B. Smith (UCLA)
Co-Investigator/Collaborators: Scott P. Layne (Co-PI, UCLA), Anne Rimoin (Co-PI, UCLA)
Title: Effects of Avian Migration and Anthropogenic Change on the Distribution and Transmission Risks of Avian Influenza
Funding Mechanism/Agency: National Institute for Allergy and Infectious Diseases (Grant # RO1 AI074059-01)
Costs: $2,513,348
Duration: 4 years (09/15/2006 - 09/14/2010)
Brief Synopsis: The project will examine the role that North American migratory birds play in the dispersion of avian influenza strains between breeding sites in Canada and the U.S. and wintering sites in Mexico and Central and South America. It will determine the geographic distribution of viral strains in relation to migratory pathways and will examine how anthropogenic environmental changes affect the prevalence and transmission dynamics of avian influenza strains between migratory and non-migratory birds associated with humans. In addition, it will examine patterns of transmission between birds and humans.

Principal Investigator: Scott P. Layne
Co-Investigator/Collaborators: Walter Boyce (Co-PI, UCD)
Title: Center for Rapid Influenza Surveillance and Research (CRISAR)
Funding Mechanism/Agency: National Institutes of Health / National Institute for Allergy and Infectious Diseases (Contract # HHSN266200700009C)
Costs: $18,878,892
Duration: 5 years (03/30/2007 - 03/29/2012)
Brief Synopsis: The project will be a collaborative and synergistic consortium of investigators at universities, government, private institutions in Alaska, California, New Mexico, and New York with established international activities. It will closely manage and undertake outstanding multi-disciplinary and collaborative surveillance and research on influenza, and actively contribute to NIAID's Pandemic Public Health Research Response Plan. Participating institutions include UCLA, UC Davis, University of Alaska Fairbanks, Wildlife Conservation Society, and Los Alamos National Laboratory.

Principal Investigator: Scott P. Layne
Co-Investigator/Collaborators: Robin M. Bush (UCD); Hong Cai (LANL); Eric L. Delwart (UCSF); Thomas B. Smith (UCLA); Nathan D. Wolfe (UCLA)
Title: High-Throughput Automated –80C Biobanking Instrument for Emerging Infections
Funding Mechanism/Agency: National Institutes of Health / National Center for Research Resources / High-End Instrumentation Grant Program S10 (in review)
Costs: $2,000,000
Duration: 1 year
Brief Synopsis: We request an automated –80C sample storage and management system configured with redundant cooling for sample protection and double rack gripper for high-throughput infectious disease research. It will provide 12 automated –80C storage compartments that are accessed by a robotic arm and automated picking mechanism operating within a –20C compartment. **Relevance** Infectious diseases are the leading cause of death worldwide and certain viruses, such as influenza, have the potential to cause explosive pandemics. Understanding factors governing virulence, transmissibility, host range as well as epidemiologic-genotypic-phenotypic associations rests on the ability to manage and test an extremely large number of viral samples as efficiently and rapidly as possible. Such crucial undertakings on much needed near real-time and high-throughput scales will depend highly on the requested biobanking instrument.

Principal Investigator: Scott P. Layne
Co-Investigator/Collaborators: Tony J. Beugelsdijk (Co-PI, LANL)
Title: UCLA High Speed, High Volume Laboratory Network for Infectious Diseases
Funding Mechanism/Agency: Congressional Appropriation through Department of Defense, Defense Threat Reduction Agency
Costs: $4,000,000 DoD FY 2008 Line Item
Duration: 1 year
Brief Synopsis: Third year of funding for project that aims to develop a new high speed, high volume (high-throughput) laboratory capability that will be linked in a network and operated by several premier institutions. The automated, networked capability will make us stronger against natural diseases and bioterrorist attacks.

CONCLUSION

Our program has made substantial progress in accomplishing all five tasks in is Statement of Work as follows. 1) The automated bioagent genotyping systems is being procured; the automated bioagent screening system has been specified in preparation for procurement. 2) The operating system and Information Technology (IT) infrastructure are being developed in parallel with the automated systems. 3) The upgrade of floor space to BSL3-enhanced specifications is underway and scheduled for completion in March 2009. 4) A quality control management program and protocols is being developed as we define, develop and/or refine assay methods. The LANL team that validates the high-throughput laboratory systems is established; the UCLA team that operates the high-throughput laboratory will be established in 2008. 5) The analysis of influenza samples from animal surveillance work is underway. This work will lead to large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses.

In addition, as described above, our FY 2006 (initial year) program has attracted major financial support from the California Office of Homeland Security (CA OHS) and National Institutes of Health / National Institute for Allergy and Infectious Diseases (NIH/NIAID). The complimentary CA OHS support greatly enhances the value of DoD's investments and greatly increases high-throughput capacity for influenza research and surge capacity for biological emergencies. The complimentary NIH/NAIAD Center-related activities will supply influenza surveillance samples from around the world and facilitate large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses.

Pandemic influenza and bioterrorism are the two top national security threats for the United States (Danzig 2003). Influenza, in particular, would easily overwhelm public health and National Guard response capabilities nationwide and downgrade military preparedness across all commands worldwide (Layne 2006). The work accomplished in this FY 2006 (initial year) and subsequent ones (option years) will result in a high-throughput laboratory network capability (with the first node at UCLA) that can be implemented and linked with other military, government and public institutions. The need for creating such a laboratory network has been widely recognized. The report *Making the Nation Safer* (National Research Council 2002) recommended the creation of a "global network for detection and surveillance, making use of computerized methods for real-time reporting and analysis to rapidly detect new patterns of disease locally, nationally, and — ultimately — internationally." In keeping with this report, the high-throughput laboratory network capability will be a critical addition to the DoD's chemical and biological defense and infectious disease programs.

In addition, the recently released Homeland Security Presidential Directive 21 (White House 2007) recognizes that "the United States must develop a nationwide, robust, and integrated biosurveillance capability, with connections to international disease surveillance systems, in order to provide early warning and ongoing characterization of disease outbreaks in near real-time." Our program addresses this Directive.

Initial analysis of avian influenza samples from surveillance work indicates that there is no universal methods for detecting (+/–) influenza viruses in samples. Detecting (+/–) and subtyping (H1–16, N1–9) by PCR in conjunction with continuously updated primer sets appears to be more sensitive than culturing in embryonated chicken eggs followed by detecting (+/–) by PCR (Runstadler 2007). With avian surveillance samples, screening with updated primers identified more positive samples than culturing followed by detecting. However, culturing followed by detection (+/–) revealed viruses in some samples that were found to be negative by detecting and screening with updated primers. These findings can be represented by a Venn diagram (see Figure 11).
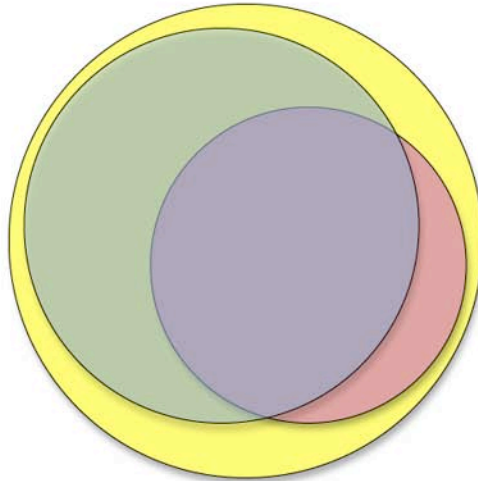
Figure 11. Venn diagram of screening with updated primers versus culturing followed by detection. All potential influenza viruses (yellow circle). Detecting and screening with continuously updated primers (green circle). Culturing followed by detecting (red circle).

For our program, these findings indicate that simultaneous automated bioagent screening and automated bioagent culturing systems will be required for robust and flexible high-throughput laboratory methods. Assembling an automated bioagent culturing system is proposed in our FY 2007 (option year 1) Statement of Work.

REFERENCES

American Society for Testing and Materials. Committee E01, E1989-98 Standard Specification for Laboratory Equipment Control Interface (LECIS). 1999. West Conshohocken, PA. http://www.lecis.org

Barr IG, Komadina N, Hurt AC, Iannello P, Tomasov C, Shaw R, et al. An influenza A(H3) reassortant was epidemic in Australia and New Zealand in 2003. Journal Medical Virology 2005;76:391–397.

Danzig RA. Catastrophic Bioterrorism — What is to be done? Center for Technology and National Security Policy. 2003. Washington, DC: National Defense University.

Francis T Jr. A New Type of Virus from Epidemic Influenza. Science 1940;91:405–407.

Holmes EC, Taubenberger JK, Grenfell BT. Heading off an influenza pandemic. Science 2005;309:989.

Holmes EC, Ghedin E, Miller N, Taylor J, Bao Y, St. George K, Grenfell BT, et al. Whole-Genome Analysis of Human Influenza A Virus Reveals Multiple Persistent Lineages and Reassortment among Recent H3N2 Viruses. PLOS Biology 2005;3(9):e300.

Homeland Security Council. National Strategy for Pandemic Influenza: Implementation Plan. May 2006. Washington, DC. http://pandemicflu.gov/

Ilyushina NA, Govorkova EA, Webster RG. Detection of amantadine-resistant variants among avian influenza viruses isolated in North America and Asia. Virology 2005;341:102–106.

Krafft AE, Russell KL, Hawksworth AW, McCall S, Irvine M, Daum LT, et al. Evaluation of PCR Testing of Ethanol-Fixed Nasal Swab Specimens as an Augmented Surveillance Strategy for Influenza Virus and Adenovirus Identification. Journal Clinical Microbiology 2005;43:1768–1775.

Lau LT, Banks J, Aherne R, Brown IH, Dillon N, Collins RA, Chan KY, Fung YWW, Xing J, Yu ACH. Nucleic acid sequence-based amplification methods to detect avian influenza virus. Biochemical Biophysical Research Communications 2004;313:336–342 .

Layne SP. Human Influenza Surveillance: the Demand to Expand. Emerging Infectious Diseases 2006;12:562 – 568.

Layne SP, Beugelsdijk TJ, Patel CKN, Taubenberger JK, Cox, NJ, Gust ID, Hay AJ, Tashiro M, Lavanchy D. A global lab against influenza. Science 2001;293:1729.

Layne SP, Beugelsdijk TJ. 1998. Laboratory Firepower for Infectious Disease Research. Nature Biotechnology 1998;16:825–829.

Layne SP, Beugelsdijk TJ. Method and Apparatus for Globally-Accessible Automated Testing. 1998. United States Patent Number 5,841,975.

Layne SP, Beugelsdijk TJ. Apparatus for Testing for Infection by a Retrovirus. 1999. United States Patent Number 5,925,514.

Layne SP, Beugelsdijk TJ. Apparatus for Automated Testing of Biological Specimens. 1999. United States Patent Number 5,968,731.

Moore C, Hibbitts S, Owen N, Corden SA, Harrison G, Fox J, Gelder C, Westmoreland D. Development and evaluation of a real-time nucleic acid sequence based amplification assay for rapid detection of influenza A. Journal Medical Virology 2004;74:619–628.

National Research Council. Committee on Science and Technology for Countering Terrorism. In: Making the Nation Safer: The Role of Science and Technology in Countering Terrorism. Chapter 3 Human and Agricultural Health Systems. 2002. Washington, DC: National Academy Press, 65–106.

Ng EKO, Cheng PKC, Ng AYY, Hoang TL, Lim WWL. Influenza A H5N1 Detection. Emerging Infectious Diseases 2005;11:1303–1305.

Payungporn S, Chutinimitkul S, Chaisingh A, Damrongwantanapokin S, Buranathai C, Amonsin A, Theamboonlers A, Poovorawan Y. Single step multiples real-time RT-PCR for H5N1 influenza A virus detection. Journal Virological Methods 2006;131:143–147.

Runstadler JA, Happ GM, Slemons RD, et al. Using RRT-PCR analysis and virus isolation to determine the prevalence of avian influenza virus infections in ducks at Minto Flats State Game Refuge, Alaska, during August 2005. Archives of Virology 2007;10:1901–1910.

U.S. Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. 2007. http://www.unh.edu/ehs/pdf/CDC-NIH-BMBL-5.pdf

White House. Homeland Security Presidential Directive/HSPD-21. October 18, 2007. http://www.whitehouse.gov/news/releases/2007/10/20071018-10.html

Xu X, Smith CB, Mungall BA, Lindstrom SE, Hall HE, Subbarao K, et al. Intercontinental Circulation of Human Influenza A(H1N2) Reassortant Viruses during the 2001–2002 Influenza Season. Journal Infectious Diseases 2002;186:1490–1493.

SUPPORTING / ADDITIONAL DATA

Pathomics Research and Surge Capacity Enhancements. The CA OHS and NIAID Center have funded two additional technologies that greatly enhance the value of our DoD supported program.

Field-use Handhelds
The CA OHS has procured 150 new devices from LANL for influenza surveillance and biological security activities (Figure 12). These field-use handhelds will be ready for deployment in February 2008. These are based on some of the latest cell phone technology and can store multi-media field surveillance data in the DAWRIN Core XML-compliant data format. They will provide standard epidemiologic questionnaires, global positioning system (GPS), voice recognition and bar-code scanner/camera functions in a rugged and waterproof housing. The population and host level data recorded with these devices will enable the "pathomics" research described above.
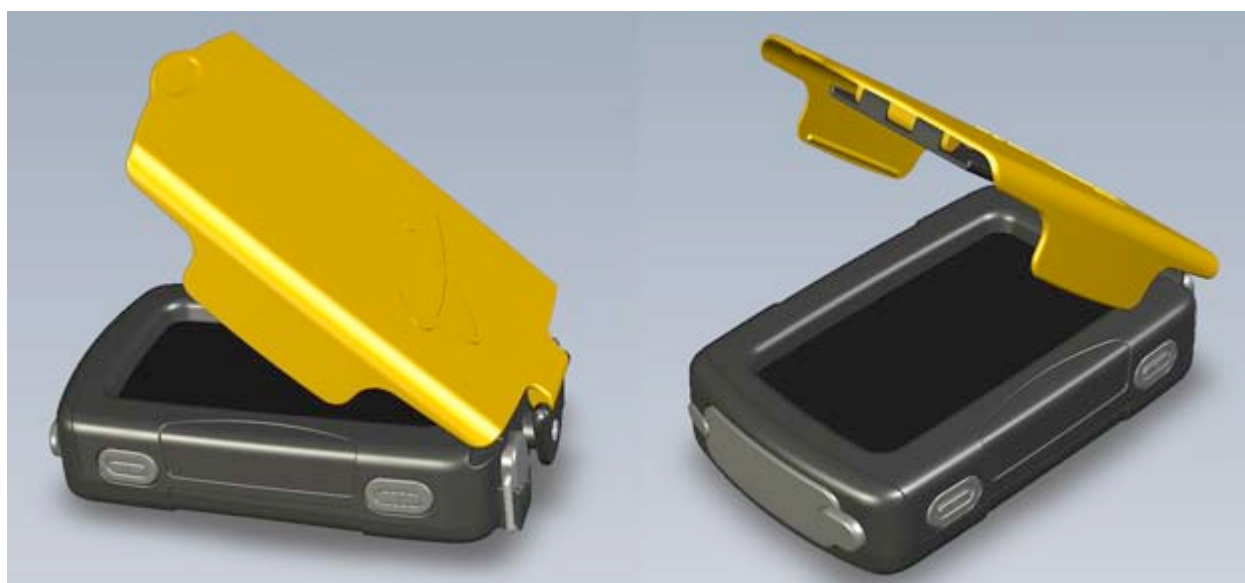
Figure 12. Field-use handhelds for capturing surveillance data. They will enable associations between field and laboratory-generated data.

Influenza Dipsticks
The NIAID Center is funding the development of field-use influenza dipstick that will yield positive-negative (+/–) results within 30 – 60 minutes (Figure 13). These simple and inexpensive molecular devices will incorporate a sample cleanup cartridge, reverse transcription and isothermal amplification cartridge, and lateral-flow detection cartridge. Based on current specifications, they will achieve a molecular threshold (sensitivity) of 100 viral genomes and molecular discrimination (specificity) that exceeds 99.9% (Cai 2006). They will provide a visual readout (i.e., the appearance of lines) and up to 10 channels for detecting different influenza types (A vs. B), subtypes (H1, H3, H5, H7, etc) and other bioagents. The dipsticks will be carried by surveillance teams in the field and used to determine whether influenza viruses are present in samples prior to their shipment to the high-throughput laboratory. The ability to detect influenza viruses in the field will facilitate rapid decision making and emergency response capabilities.
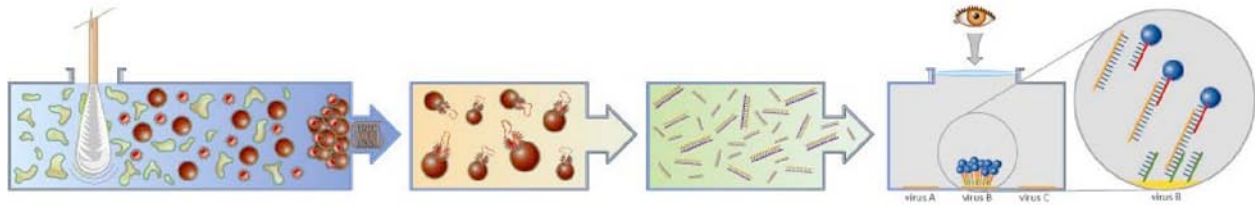
Figure 13. Components and steps for the influenza dipstick.

Other Surveillance Activities
In addition to the NIAID Center, which is currently collecting 20,000 samples per year worldwide, the UCLA Department of Ecology and Institute of the Environment have a well established animal surveillance program that spans North America, South America, the Caribbean and western Africa. This parallel program takes advantage of hundreds of bird banding stations on these continents and is currently collecting over 20,000 samples per year. These samples are associated with time, place and species identifiers and consist of cloacal swabs preserved in ethanol and/or viral transport media.

In summary, our High Speed, High Volume Laboratory Network for Infectious Diseases program has access currently to over 40,000 samples per year for viral pathomics research.

APPENENDICES

• Layne SP. Human Influenza Surveillance: the Demand to Expand. Emerging Infectious Diseases 12, 562 – 568 (2006).

• Song J, Gans J, Wolinsky M, Li P, Curd E, Detter C, Hong C. Algorithms for Designing the Minimal Set of Multiplexed Degenerate Universal Tagged Primers for RNA Virus Detection. (Submitted to BIOCOMP'08 Conference).

• UCLA Request For Proposals (RFP). LIMS Evaluation & Recommendation for High-Throughput Flu Lab Pages 1 – 16 (November 29, 2007).

• LANL. High-Throughput Laboratory Network (HTLN): General IT Requirements for HTLN Instrumentation / Version 1.1 (January 23, 2008).

• UCLA Request For Proposals (RFP). Genotyping System for University of California, Los Angeles. Pages 1 – 35 (March 6, 2008).

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Human Influenza Surveillance: the Demand to Expand

Scott P. Layne*

The World Health Organization Influenza Program is one of the best developed and longest running infectious disease surveillance systems that exists. It maintains a worldwide watch of influenza's evolution to assist delivery of appropriately formulated vaccines in time to blunt seasonal epidemics and unpredictable pandemics. Despite the program's success, however, much more is possible with today's advanced technologies. This article summarizes ongoing human influenza surveillance activities worldwide. It shows that the technology to establish a high-throughput laboratory network that can process and test influenza viruses more quickly and more accurately is available. It also emphasizes the practical public health and scientific applications of such a network.

Influenza strikes persons in developing and industrialized countries alike and is capable of killing healthy persons of all ages. Among the hardest hit are infants <1 year of age and adults >65. During any given year, influenza epidemics kill 500,000–1,000,000 persons globally, and an unpredictable pandemic is capable of killing millions (*1*). Yet death rate statistics alone do not capture the full impact of influenza; it causes many hospitalizations, secondary bacterial pneumonias, and middle ear infections in infants and young children (*2*). Worldwide, literally tons of antimicrobial drugs are used to treat these complications, and the economic consequences are enormous. For large populations, the only way to deter influenza is to administer vaccines targeted against ever-mutating strains (*3*).

Current Surveillance

The World Health Organization (WHO) Influenza Program was established in 1952 to assist with public health threats associated with influenza. Today, its network of 112 national centers in 83 countries collects ≈160,000 samples each year from 600 to 1,200 million persons with influenza. As shown in Figure 1, the centers screen sam-

ples for influenza viruses and type- (A versus B) and subtype- (e.g., A/H1N1, A/H3N2) relevant samples (*4*). Certain influenza-positive samples are then forwarded to 1 of 4 WHO collaborating centers for further immunologic and genetic characterizations. Twice a year, WHO organizes a formal meeting with its collaborating center directors to review information on circulating influenza strains. This advisory committee identifies circulating strains that new vaccine formulations should target. Because influenza epidemics peak during the winter months, the committee offers its recommendations in February and September for the Northern and Southern Hemispheres, respectively. The findings are then reviewed by national health authorities who approve, and occasionally amend, implementation of the recommended vaccine strains (*5*). Surveillance for influenza requires global and national monitoring for both virus and disease activity to determine when, where, and which influenza viruses are circulating in the United States and globally, to determine the intensity and impact of influenza activity on defined health outcomes and identify unusual or severe outbreak, and to detect the emergence of novel influenza viruses that may cause a pandemic.

The Centers for Disease Control and Prevention's (CDC) WHO Collaborating Center for Reference and Research on Influenza supplies standardized reagents and test kits to all national centers for detecting influenza A and B strains, subtyping strains, and determining whether sample strains are immunologically related to recent vaccine strains. Typical circulating strains and atypical ones that appear to differ from vaccine strains are forwarded to 1 of 4 collaborating centers (in the United States, United Kingdom, Australia, and Japan) for further characterization (*5*). During the 2004–2005 influenza season, CDC's center in Atlanta received ≈3,500 strains from domestic and foreign surveillance. A partial summary of laboratory methods used is outlined below.

In most situations, 6–10 serum samples are used to compare sample strains against vaccine and reference strains, and during any given influenza season, >99% of

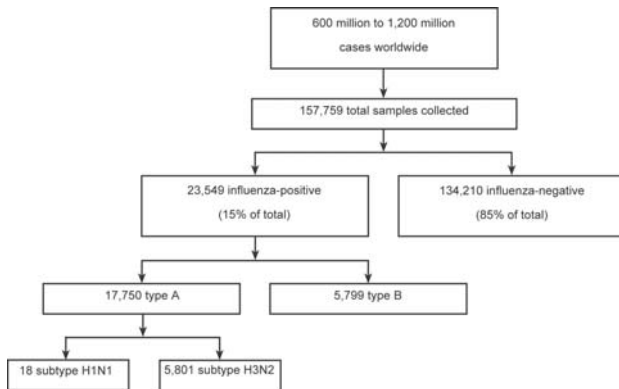*University of California at Los Angeles, Los Angeles, California, USA

Figure 1. Global influenza surveillance for the 2004–2005 season. Respiratory samples were collected from persons with febrile respiratory illness worldwide. Approximately 15% of samples were influenza positive. Note that only some of the type A viruses were subtyped. Data are from the Centers for Disease Control and Prevention website (http://www.cdc.gov/flu/weekly/weeklyarchives2004-2005/04-05summary.htm).

sample strains are successfully matched by routine hemagglutination inhibition (HI) assays. Laboratory workers replicate sample strains in cell cultures or fertile eggs and adjust the resulting viral stocks to a standard hemagglutinin titer. They then use HI assays to determine whether sample strains are immunologically related (i.e., cross-reactive) to recent vaccine strains. Typing sera are added to wells in a series of 2-fold dilutions, and after the reactions stabilize, laboratory workers score assay wells (positive or negative) by looking for agglutinated erythrocytes (positive) that do not form buttons at the bottom of plates versus non-agglutinated cells (negative) that do form buttons. For sample strains that behave as variants, laboratory workers inject them into ferrets to produce a strain-specific antiserum. When the new antiserum is ready, HI assays are again performed as above. If the new sera show significant gaps in cross-reactivity (usually defined as a 4-fold difference between sample and vaccine strains), they are incorporated into the routine laboratory set and used to look for new epidemic strains. Examples of strains that were identified as variants include A/H3N2/Sydney, which spread rapidly among persons in 1997, and A/H5N1/Hong Kong, which jumped from fowl to humans in 1997. During the 2004–2005 influenza season, ≈3,500 HI assays were performed.

During the 2004–2005 influenza season, >600 cleaved hemagglutinin (HA1) domain sequences were analyzed. In this process, laboratory workers extract viral RNA from samples, convert viral RNA to cDNA, amplify cDNA with polymerase chain reaction (PCR) primers, and analyze DNA products with capillary array sequencers. They then compare sample strain sequences against vaccine and ref-

erence strain sequences to determine their phylogenetic relationships.

During the 2004–2005 influenza season, ≈350 neuraminidase (NA) sequences were analyzed to determine their phylogenetic relationships. For this process, laboratory workers follow similar preparative steps used for HA1 and then use multiple PCR primers to sequence the entire gene segment.

During the 2004–2005 influenza season, ≈3,500 single nucleotide polymorphism (SNP) profiles were analyzed from circulating strains. For this analysis, laboratory workers follow similar preparative steps used for HA1 and then use pyrosequencing to detect SNP that confer resistance to the antiviral drugs amantadine and rimantadine.

During the 2004–2005 influenza season, ≈120 whole genomes were sequenced from circulating strains. In this process, laboratory workers extract viral RNA from samples, convert viral RNA to cDNA, amplify cDNA with PCR primers, and analyze DNA products with capillary array sequencers. Amplification of all 8 gene segments (PB1, PB2, PA, HA, NP, NA, M1/M2, NS1/NEP) that have a combined length of ≈13.6 kb requires ≈30 type- and subtype-specific PCR primers.

These activities give ≈6 months to vaccine manufacturers on either side of the equator for scale up, production, and distribution. These activities also give healthcare services another 3 months to administer the ≈250 million doses of trivalent vaccine that are used globally. Despite its sophistication and scale, however, the WHO Influenza Program has several shortcomings (6).

First, surveillance gaps exist in many parts of the world for a variety of reasons, including limited funding, lack of infrastructure support for surveillance teams and preserving influenza samples, and intentional underreporting at the national level (7). Second, current laboratory methods for characterizing influenza are time-consuming and labor-intensive and, as a result, relatively few viral strains undergo definitive phenotyping and genotyping assays (5,8,9). During the 2004–2005 influenza season, for example, the 4 WHO collaborating centers analyzed ≈6,000 strains, representing only 1 sample per 100,000 influenza cases worldwide (Figure 1). Far fewer strains from domestic poultry and swine or from wild aquatic birds, which are thought to serve as precursors for pandemic strains, are analyzed comprehensively in a given year (10).

Third, with current methods, it can take weeks to months to generate laboratory data on influenza samples and understand their significance (Appendix available online at http://www.cdc.gov/ncidod/EID/vol12no04/05-1198_app.htm). Such prolonged times can impede vaccine strain selection activities. For example, the vaccine administered throughout North America in 1997 provided inadequate protection against the A/H3N2 Sidney strain

that spread rapidly from Asia and Australia (*11*). Public health officials ascribed the poor match between circulating strains and vaccine strains to many factors including 1) less than optimal surveillance, 2) time required to prepare and ship isolates, 3) lag time in laboratory testing with current manual methods, and 4) rapid spread of the Sydney variant.

Although the program has a remarkably good track record, it did not detect the Sydney variant in time to include it in the vaccine before the epidemic. Failure to detect an emerging influenza virus could prove disastrous should it be a novel strain with pandemic potential (*12*). The 1918 influenza pandemic is the biggest infectious disease catastrophe on record, topping even the medieval Black Death. Within months after the initial outbreak, the A/H1N1 virus struck 500 million persons and killed 40–50 million worldwide when the total population was only 2 billion (*13*). Subsequent pandemics, brought about by a shift to A/H2N2 in 1957 and A/H3N2 in 1968, had far lower death rates.

Between March and May of 1997, an outbreak of avian A/H5N1 in Hong Kong killed a child who was otherwise healthy and thousands of chickens (*14*). For the next 6 months, no new cases appeared. Between November and December of 1997, avian A/H5N1 infected 17 people and killed 5 of them (*15,16*). Confronted with a case-fatality ratio of 33%, health authorities took quick action and opted to destroy all 1.5 million chickens in Hong Kong. It took nearly 6 months to identify the index case; events transpired so quickly that manual laboratory methods were unable to generate all of the information that was needed.

Since 2004, avian A/H5N1 has caused additional outbreaks throughout Asia, resulting in >60 human deaths in Vietnam, Thailand, Cambodia, and Indonesia and the destruction of 150 million birds (*17*). In 2005, through a combination of wild bird migrations and farming practices, highly pathogenic avian influenza spread to northern China, Mongolia, Tibet, Kazakhstan, and Russia (*18*). At the time of this writing, it had further spread to several European countries (Turkey, Romania, and Greece) and threatened to spread to other continents, including Africa and North America through avian flyways.

The potential of avian A/H5N1 to cause a global human pandemic is uncertain because it cannot be predicted with current knowledge (*19*). Nevertheless, the anticipated economic, social, and political consequences are enormous (*20,21*). Therefore, we face a compelling demand to expand the current influenza surveillance system (*6,22*).

High-throughput Network

In August 2004, the US Department of Health and Human Services released a draft of its Pandemic Influenza Response and Preparedness Plan. The plan's surveillance annex offered specific recommendations for system enhancements and next steps (*5*). Many of these enhancements could be achieved by developing a high-throughput laboratory network that would expand the capabilities of the existing WHO collaborating centers on influenza (*6,22*). With such enhancements, WHO national centers would be able to collect samples from people with febrile respiratory illnesses, record epidemiologic observations, and send samples directly to the high-throughput network. At each site, high-throughput automated systems would collaborate, and epidemiologic observations and test results would appear in the laboratory's web-enabled database for analysis within days (*23*). Internet-based capabilities would allow WHO national centers to examine their own data and improve surveillance in an iterative manner (Figure 2). In tracking changes in epidemic strains, the new system would facilitate nonbiased proportional sampling of persons with febrile respiratory illnesses in an iterative fashion. In detecting the emergence of novel strains with pandemic potential, the new system would facilitate the use of rapid and more sensitive methods.

The plan integrates available biologic, engineering, and informatic technologies into a networked capability and
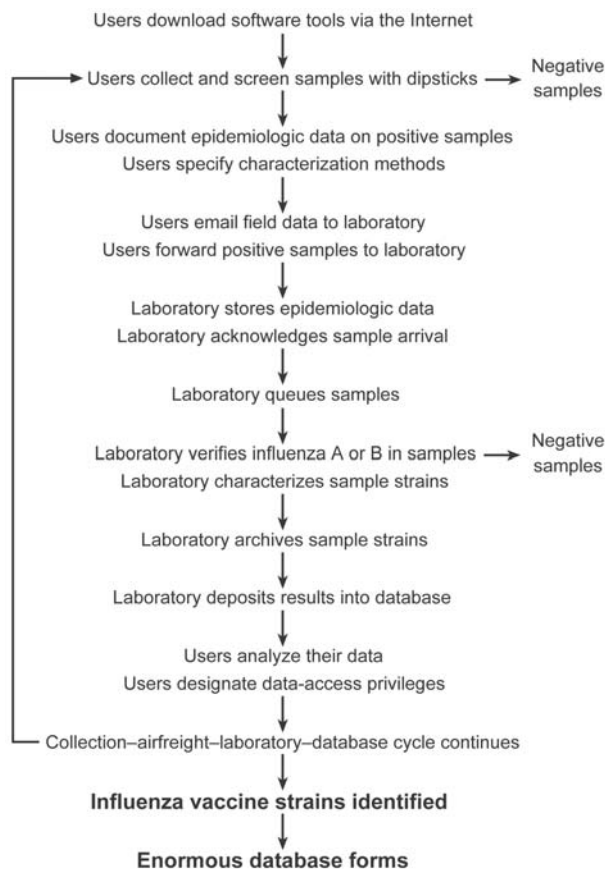

Figure 2. Flow chart for utilizing the high-throughput laboratory network.

makes them available through the Internet (*23*). Influenza is well suited to this approach because of its obvious public health implications, but also because a well-established infrastructure that includes global surveillance, standardized laboratory methods, surveillance-based recommendations, and targeted vaccines already exists (*6,22*). Key elements are shown in Figure 2.

Platform-independent software that facilitates influenza surveillance would be provided by the high-throughput network (*23*). Internet-based tools would manage laboratory access, epidemiologic questionnaires, testing instructions, sample submission, data analysis, and data privileges. The release of data to other users or entities would be controlled by the submitting organization or authority. More importantly, the WHO committee and national health authorities that recommend and review vaccine strains and antiviral drugs would have access to all the data.

On average, 1 of every 6 samples collected from persons with febrile respiratory illnesses contains influenza A or B viruses (*24*). The remainder contain other viral and bacterial pathogens. To expedite data collection, surveillance teams could use influenza dipsticks to screen samples on the spot. Several companies make diagnostic kits for influenza A and B; although these tests have certain disadvantages (limited sensitivity and specificity of immunoassays), their underlying technologies can form the basis for improved influenza screening and sampling devices (*2*). Such influenza dipsticks—or even portable PCR-based assays—would make it easier for teams in the field to screen out negative samples and focus on documenting epidemiologic information on positive ones.

An epidemiologic questionnaire would be provided to surveillance teams, with a menu that covers key questions (*23*). What are the collection date and location? Who is the host (human versus animal)? What is the age of the host? What is the physiologic source of the sample? What is the observed or reported severity of illness? What is the observed or reported outcome of illness? Which influenza vaccine or antiviral agents were administered? What are the likely exposures? Is there any recent travel history? The questionnaire would run on inexpensive handheld devices (e.g., personal digital assistants) or cell phones. Bar codes would be used to link samples to their corresponding questionnaires. Completed questionnaires would be sent by email to the high-throughput laboratory network, where questionnaire and laboratory data would form the basis for seeking associations on factors that influence virulence, transmissibility, and host range (*19*).

Current high-throughput automated laboratory systems are capable of operating 24 hours a day. At each networked site, epidemiologic questionnaires and instructions would arrive by the Internet, and bar coded samples would arrive by air freight. Larger sites could operate systems for genotyping, phenotyping, replicating, and archiving influenza viruses. Smaller sites could operate systems for genotyping and archiving viruses. In serving as resources, each site would provide reagents and supplies for analyzing all influenza subtypes. They would also perform control assays on a daily basis and maintain a quality assurance program, the documentation of which would be stored in the database. Automated laboratory methods would build upon manual methods currently in use and, because they can reduce working (liquid) volumes by at least 5- to 10-fold, they would enable economies of scale (*23,25*).

Genotyping systems would have flexibility to sequence all 8 RNA segments or any individual segment from influenza viruses. The various steps performed would include transcription viral RNA into cDNA, selection of optimal PCR primers, amplification of DNA by PCR, and analysis by capillary array DNA sequencers (*26–28*). Influenza viruses are often propagated in cell cultures or embryonated eggs to obtain enough viral RNA for sequencing. Newer methods that avoid this growth step, however, would facilitate direct high-throughput analysis of native samples from surveillance, including active samples preserved by cold chain as well as inactive samples preserved by ethanol fixation (*29*).

Phenotyping systems would conduct HI and neuraminidase inhibition (NI) assays. HI assays are easily adaptable to automation, but they require relatively large quantities of virus and typing sera (*8*). To overcome this drawback, automated methods that use flow cytometry are under development (*30*). They work by attaching monoclonal or polyclonal typing sera to a set of color-coded (multiplexed) beads and detecting the interaction of influenza with such beads. A high-throughput system that performs HI assays in parallel with flow cytometer–based assays, for example, may offer the best means to test and validate improved influenza serotyping methods. NI assays are also easily adaptable to automation, particularly newer ones that use a chemiluminescent sialic acid substrate instead of a fluorogenic substrate (*31*). They work by mixing substrate with neuraminidase from sample strains and measuring the chemiluminescent signal over time. When performed over a range of inhibitor or antiviral drug (oseltamivir and zanamivir) concentrations, they enable the determination of the 50% inhibitory concentration (IC_{50}) for individual drugs and strains (*32*).

A replicating system would verify that influenza A and B antigens are present in samples and set aside negative ones (*33*). Positive samples would be injected into cell cultures or embryonated eggs and, several days later, automatically harvested, assayed for HA titers, and adjusted to uniform concentrations. A part of this fresh stock would then go to the long-term storage system.

Archiving systems would store influenza samples for an extended time. The archiving system would take stocks from the replication system and place them into modular bar-coded storage containers, which would then be placed into freezers. Every step in the storage and retrieval process would be recorded by bar code scanners and managed by an inventory tracking program (23).

Expanded Surveillance

Influenza virus evolves through a combination of point mutations (drifts) and reassortment events (shifts) in its gene segments. For vaccine strain selection, laboratory methods for characterizing influenza have focused primarily on changes in hemagglutinin and neuraminidase (and to a much lesser extent on the M2 ion channel protein) because immunity against these surface proteins is protective (2). The emphasis on immune-inducing proteins is clearly practical, but it may overlook changes in the remaining gene segments (26).

Whole-genome sequencing and phylogenetic analysis of 156 A/H3N2 viruses that infected humans in New York from 1999 to 2004 shows 2 substantial findings (28). The first is that multiple influenza strains co-circulated in humans over time, with strains falling into 1 of 3 distinct clades. The second is that mixing between these clades occurred over short intervals of time, resulting in at least 4 reassortment events among the strains analyzed (28). One such reassortment event (shift), rather than a point mutation (drift), appears to explain the emergence of the A/Fujian/411/2002-like strain that caused an epidemic during the 2003–2004 influenza season. Similar findings on A/H3N2 viruses that infected humans in Australia and New Zealand from 2003 to 2004 have also been reported (27). In this instance, swaps of neuraminidase and 3 internal genes (NS1/NEP, NP, M1/M2) were found. Both independent findings show that influenza A virus is less restricted than previously believed and that reassortment events can occur without warning. Altogether, these new findings underscore the importance of rapid, whole-genome analysis for future influenza surveillance (28).

The high-throughput laboratory network would give rise to 3 domains of associated data from surveillance (34). Epidemiologic data would pertain to dates, locations, hosts, outcomes, histories, and exposures. Genotypic data would pertain to the exact sequence of nucleotides in all 8 viral RNA segments. Phenotypic date would pertain to immunologic pedigrees (HI titers) and antiviral drug sensitivities (IC_{50}) of sample strains. Some practical public health and scientific uses of such organized data follow.

New influenza vaccines are often introduced after 3 criteria have been met (25). First, a new strain is identified by laboratory-based methods. Second, geographic spread of the new strain is associated with human illness. Third, the most recent influenza vaccine stimulates a reduced immunologic response to the new strain. Given these criteria, the high-throughput laboratory network would help in 2 ways. It would provide faster information for vaccine strain selection, potentially saving 1–2 months in vaccine delivery. It would also continuously monitor for the emergence of escaping influenza strains and guide critical decisions to update pandemic vaccines or use them in combination with limited supplies of antiviral drugs (19). Researchers and drug companies are developing modern methods (based on reverse genetics and cell cultures, for example) to manufacture influenza vaccines that could cut delivery times in half (2,35). Within the next few years, these new methods, in combination with a high-throughput network, could save additional vaccine delivery time and save lives (6,19).

Fifteen hemagglutinin (H1–H15) and 9 neuraminidase (N1–N9) subtypes diverge by as much as 50% in their overall amino acid composition. Within each subtype, smaller amino acid substitutions (drifts) that enable influenza viruses to evade preexisting immunity exist (2,3). Although sequencing influenza viruses is useful for understanding viral mixing and evolution, it cannot delineate how immunologic (i.e., drift and shift) variants relate to one another at the amino acid and RNA coding levels. To develop such understanding, a large base of phenotypic data must be associated with its corresponding genotypic data. For each receptor subtype, phenotypic data would consist of HI titers and genotypic data would consist of RNA sequences from the same virus. Building a rough association matrix would be the first step in understanding how variants relate to one another at the amino acid and RNA levels.

Subsequently, a more complete association matrix would be used to develop models that could predict whether viral strains are immunologically related from sequences alone. Such efforts could help develop influenza vaccines that protect against a wider range of variants and establish a more fundamental molecular basis for influenza surveillance (25).

Researchers have proposed using antiviral drugs such as oseltamivir to halt an avian influenza outbreak in humans (36,37). The strategy would require stockpiling millions of doses and administering them to persons in the epicenter and surrounding areas within weeks. Immediate recognition of the outbreak and rapid surveillance to determine its size would be essential. Drug-resistant avian influenza viruses would likely emerge at some point, representing a potential threat to emergency control efforts, and health authorities would need real-time information on where the viruses were found and how many of them existed (38). Such emergency interventions would generate thousands of samples for laboratory analysis within days.

Given current laboratory surge capacity, a high-throughput laboratory network may be the only feasible means to meet the challenge.

Implementation

The spreading avian A/H5N1 outbreak poses serious threats to the health, economy, and security of the world (*22*). It has motivated political leaders and health officials to increase financial support for influenza surveillance and to seek agreements and incentives that promote information sharing and international cooperation (*39*). Effective measures will require real-time, accurate, and comprehensive information to make rapid public health decisions.

The first 2 sites in a high-throughput laboratory network could be up and running in 12–18 months at a cost of $15 million. It would generate epidemiologic, genotypic, and phenotypic data as described in this article. With available technologies and methods, each site would be capable of analyzing up to 10,000 samples per year, a substantial improvement over current capabilities. Implementing multiple sites worldwide (at the 4 WHO collaborating centers, for example) would enable regional collaborations and help encourage the timely sharing of samples and information (*40*).

Human history shows that an influenza pandemic is years overdue (*41*). Moreover, whole-genome sequencing of influenza virus shows dynamic RNA segments that are capable of epidemiologically meaningful reassortment events (*27,28*). Whether avian A/H5N1 will be the precursor strain is unknown. However, we must expand, speed up, and connect human and animal surveillance efforts today, which must be matched with an expanded capacity to produce and deliver influenza vaccines worldwide.

Dr Layne is an associate professor of epidemiology at the University of California at Los Angeles School of Public Health in Los Angeles, California. His primary research interests include viral diseases such as influenza, bioterrorism preparedness, and laboratory and informatic tools to deal with these threats.

References

1. Ghendon, Y. Influenza—its impact and control. World Health Stat Q. 1992;45:306–11.
2. Nicholson KG, Wood JM, Zambon M. Influenza. Lancet. 2003;362:1733–45.
3. World Health Organization. Influenza vaccines. Wkly Epidemiol Rec. 2002;77:229–40.
4. World Health Organization. WHO Global Influenza Programme: survey on capacities of national influenza centres, January–June 2002. Wkly Epidemiol Rec. 2002;77:349–56.
5. Department of Health and Human Services. Pandemic Influenza Response and Preparedness Plan, Annex 4: Surveillance. [cited 2006 Feb 17]. Available from http://www.hhs.gov/pandemicflu/plan
6. Layne SP, Beugelsdijk TJ, Patel CKN, Taubenberger JK, Cox NJ, Gust ID, et al. A global lab against influenza. Science. 2001;293:1729.
7. World Health Organization. WHO report on global surveillance of epidemic-prone infectious diseases. Geneva: The Organization; 2000.
8. Kendal AP, Pereira MS, Skehel J. Concepts and procedures for laboratory-based influenza surveillance. Geneva: World Health Organization; 1982.
9. Hay AJ, Wolstenholme AJ, Skehel JJ, Smith MH. The molecular basis of the specific anti-influenza action of amantadine. EMBO J. 1985;4:3021–4.
10. Webster, RG. Influenza: an emerging disease. Emerg Infect Dis. 1998;4:436–41.
11. Centers for Disease Control and Prevention. Update: influenza activity—United States and worldwide, 1997–98 season, and composition of the 1998–99 influenza vaccine. MMWR Morb Mortal Wkly Rep. 1998;47:280–4.
12. World Health Organization. WHO global influenza preparedness plan. Geneva: The Organization; 2005.
13. Reid AH, Taubenberger JK. The 1918 flu and other influenza pandemics: "over there" and back again. Lab Invest. 1999;79:95–101.
14. Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, et al. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science. 1998;279:393–6.
15. Yuen KY, Chan PKS, Peiris M, Tsang DNC, Que TL, Shortridge KF, et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet. 1998;351:467–71.
16. Claas ECJ, Osterhaus ADME, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet. 1889;351:472–7.
17. World Health Organization Global Influenza Program Surveillance Network. Evolution of H5N1 avian influenza viruses in Asia. Emerg Infect Dis. 2005;11:1515–21.
18. World Health Organization. Avian influenza—new areas with infection in birds—update 34. Communicable Disease Surveillance and Response Unit. [cited 2006 Feb 17]. Available from http://www.who.int/csr/don/archive/disease/influenza/en
19. Holmes EC, Taubenberger JK, Grenfell BT. Heading off an influenza pandemic. Science. 2005;309:989.
20. Meltzer MI, Cox NJ, Fukuda K. The economic impact of pandemic influenza in the United States: priorities for intervention. Emerg Infect Dis. 1999;5:659–71.
21. Osterholm MT. Preparing for the next pandemic. N Engl J Med. 2005;352:1839–42.
22. Fouchier R, Kuiken T, Rimmelzwaan G, Osterhaus, A. Global task force for influenza. Nature. 2005;435:419–20.
23. Layne SP, Beugelsdijk TJ. Laboratory firepower for infectious disease research. Nat Biotechnol. 1998;16:825–9.
24. Centers for Disease Control and Prevention. Update: influenza activity—United States and worldwide, 2003–04 season, and composition of the 2004–05 influenza vaccine. MMWR Morb Mortal Wkly Rep. 2004;53:547–52.
25. Cox NJ. Expanding the worldwide influenza surveillance system and improving the selection of strains for vaccines. In: Layne SP, Beugelsdijk TJ, Patel CK, editors. Firepower in the lab: automation in the fight against infectious diseases and bioterrorism. Washington: Joseph Henry Press; 2001. p. 47–53.
26. Xu X, Smith CB, Mungall BA, Lindstrom SE, Hall HE, Subbarao K, et al. Intercontinental circulation of human influenza A(H1N2) reassortant viruses during the 2001–2002 influenza season. J Infect Dis. 2002;186:1490–3.

27. Barr IG, Komadina N, Hurt AC, Iannello P, Tomasov C, Shaw R, Et al. An influenza A(H3) reassortant was epidemic in Australia and New Zealand in 2003. J Med Virol. 2005;76:391–7.

28. Holmes EC, Ghedin E, Miller N, Taylor J, Bao Y, St. George K, et al. Whole-genome analysis of human influenza A virus reveals multiple persistent lineages and reassortment among recent H3N2 viruses. PLoS Biol. 2005;3:e300.

29. Krafft AE, Russell KL, Hawksworth AW, McCall S, Irvine M, Daum LT, et al. Evaluation of PCR testing of ethanol-fixed nasal swab specimens as an augmented surveillance strategy for influenza virus and adenovirus identification. J Clin Microbiol. 2005;43:1768–75.

30. Yan X, Schielke EG, Grace KM, Hassell C, Marrone BL, Nolan JP. Microsphere-based duplexed immunoassay for influenza virus typing by flow cytometry. J Immunol Methods. 2004;284:27–38.

31. Buxton RC, Edwards B, Juob RR, Voytab JC, Tisdalea M, Bethella RC. Development of a sensitive chemiluminescent neuraminidase assay for the determination of influenza virus susceptibility to zanamivir. Anal Biochem. 2000;280:291–300.

32. Mungall BA, Xu X, Klimov A. Surveillance of influenza isolates for susceptibility to neuraminidase inhibitors during the 2000–2002 influenza seasons. Virus Res. 2004;103:195–7.

33. Ziegler T, Hall H, Sánchez-Fauquier A, Gamble WC, Cox NJ. Type- and subtype-specific detection of influenza viruses in clinical specimens by rapid culture assay. J Clin Microbiol. 1995;33:318–21.

34. National Research Council. Human and agricultural health systems. In: Making the nation safer: the role of science and technology in countering terrorism. Washington: National Academies Press; 2002. p. 65–106.

35. Webby RJ, Perez DR, Coleman JS, Guan Y, Knight JH, Govorkova EA, et al. Responsiveness to a pandemic alert: use rapid development of influenza vaccines. Lancet. 2004;363:1099–103.

36. Ferguson NM, Cummings DAT, Cauchemez S, Fraser C, Riley S, Meeyai A, et al. Strategies for containing an emerging influenza pandemic in Southeast Asia. Nature. 2005;437:209–14.

37. Longini IM Jr, Nizam A, Xu S, Ungchusak K, Hanshaoworakul W, Cummings DAT, et al. Containing pandemic influenza at the source. Science. 2005;309:1083–7.

38. Kiso M, Mitamura K, Sakai-Tagawa Y, Shiraishi K, Kawakami C, Kimura K, et al. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. Lancet. 2004;364:759–65.

39. US Department of State. U.S. launches international partnership on avian and pandemic influenza (2005 Sep 22). [cited 2006 Feb 9]. Available from http://www.state.gov/r/pa/prs/ps/2005/53865.htm

40. Stöhr K. The global agenda on influenza surveillance and control. Vaccine. 2003;21:1744–8.

41. Taubenberger JK, Layne SP. Diagnosis of influenza virus: coming to grips with the molecular era. Mol Diagn. 2001;6:291–305.

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Algorithms for Designing the Minimal Set of Multiplexed Degenerate Universal Tagged Primers for RNA Virus Detection

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ABSRACT

RNA viruses, such as HIV and influenza viruses, are inherently hyper-variable and thus present a great challenge for nucleic acid-based detection and disease diagnosis. Most of the published molecular signatures for detection were designed and tested against a limited collection of sequences. They often have low coverage and have quickly become obsolete for the detection of clinical or field samples as the targeted viruses evolve and new strains emerge. The key to meeting this challenge is a rapid assay design capability that can routinely update detection assays based on current knowledge of RNA virus sequence diversity. To this end, we have developed a semi-automated signature design pipeline. It exhaustively searches the entire target sequence space to find a minimal set of multiplexed, degenerate, non-overlapping, universal tagged primers and/or probes for maximal coverage, increased sensitivity and specificity for different types or subtypes. It also regularly evaluates existing detection assays to identify potential false positive or false negative results.

1

Introduction

  Nucleic acid-based assay has become a popular method of detection for RNA viruses due to its superior sensitivity, specificity and reproducibility. Detection of HIV using nucleic acid tests is standard practice to screen blood and blood products, and monitor the viral load during anti-viral treatment (*Constantine & Zhao 2005; Constantine & Zink 2005*). These tests are also well suited to detect new primary or acute HIV infections shortly after individuals have been initially infected. During the early acute stage of infections, the viral RNA is the earliest marker to show up while a detectable level of HIV antibodies has yet to develop. There is mounting evidence suggesting that a significant portion of HIV transmissions occur during this early stage of infection. So early detection of HIV-infected individuals is critical in slowing the spread of HIV. Detection and differentiation of the major flu subtypes, particularly H5N1, has also attracted a great deal of attention from the possibility of a potential pandemic as the avian flu H5N1 continues to spread worldwide and more human infections are being reported(*Auewarakul et al. 2007; Wu et al. 2008*). Early detection and diagnosis of flu infection and the accurate monitoring of the spread of avian flu will be critical for stopping the spread of avian flu viruses with timely quarantine of infected human or culling the infected domestic birds.

  Despite the advantages of these tests, nucleic acid-based detection of HIV and influenza viruses, however, has been greatly complicated by the extreme genetic diversity of these viruses. As RNA viruses, they are inherently hyper-variable because RNA polymerases, which replicate the viral genome, lack proof-reading and error-editing capabilities that are found in DNA polymerases (*Steinhauer & Holland 1987*). RNA viruses evolve at a rate 1 million times that of eukaryotic DNA genomes (*Mansky 1998*). This rapid evolution presents a great challenge for nucleic acid-based molecular tests in term of signature design. In order to deal with this high sequence variability, degenerate primers can be used to detect most, if not all, known strains. Degenerate primers have an obvious advantage over regular unique primers in that they can cover all possible variants (strains) of viruses including those that are currently unknown, which is very important given the high rate of mutation. Degenerate primers have been used in both pathogen detection and the cloning of homologous genes (*Chen & Plagemann 1995; Ehlers et al. 1999; Rose et al. 2003; Linhart & Shamir 2005; Jabado et al. 2006*). However, degeneracy does have one drawback: it often results in decreased sensitivity of the assays, as a highly degenerate primer set has very few primer species that precisely match the target sequence (*Jabado et al. 2006*). This problem has limited the level of degeneracy that can be used in a single primer. To circumvent this problem, a set of multiplexed primers that can detect all or a majority of the known target sequences with the minimum amount of degeneracy is therefore required.

  While multiplexed real-time RT-PCR detections of HIV and Flu viruses have been developed (*Daum et al. 2002; Gibellini et al. 2006; Coleman et al. 2007; Wu et al. 2008*), they mostly use unique primers/probes to detect only the targets for which they were designed (*Coleman et al. 2007*) and thus have very low coverage (*Wu et al. 2008*) when tested against current sequences. Currently, there are no standard primer/probe sets available for HIV and influenza detection. None of the existing tools for designing multiplexed primers meet our signature design needs in terms of coverage, specificity and sensitivity. Here we report the development of a semi-automated signature design pipeline. It will exhaustively search the entire

sequence space to find a minimal set of multiplexed, degenerate, non-overlapping, universal tagged primers and/or probes for maximal coverage, increased sensitivity and specificity for different types or subtypes. It is also being used to regularly evaluate our existing detection signatures for timely update to identify gaps in detection coverage.

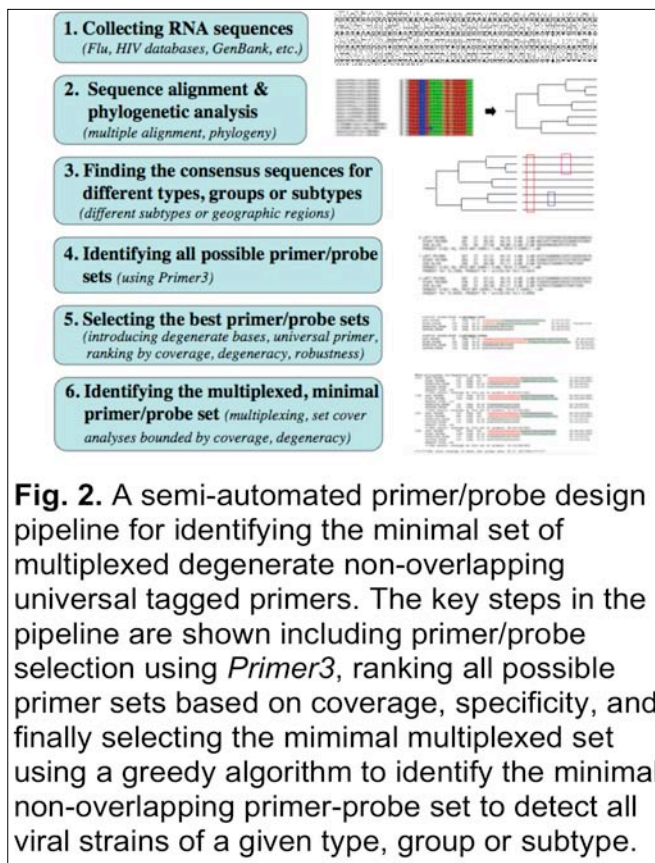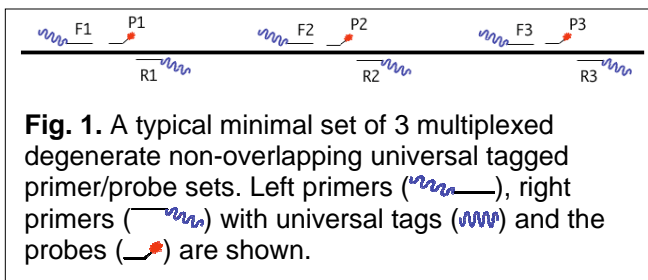## Algorithms and Implementation

The goal of our primer/probe design pipeline is to find the minimal set of multiplexed degenerate non-overlapping universal tagged primers/probes (**Fig. 1**) that will allow detection of all known strains of a given type or subtype. To achieve this goal, several new algorithms have been developed for: (1) introducing degenerate bases into the primer/probe to

Fig. 1. A typical minimal set of 3 multiplexed degenerate non-overlapping universal tagged primer/probe sets. Left primers (〰〰━), right primers (━〰〰) with universal tags (〰〰) and the probes (━●) are shown.

maximize the coverage, (2) checking robustness of each primer/probe set for false positives and potential non-specific hybridization to the background (*e.g.,* human DNA), (3) ranking the primer/probe sets based on their coverage, (4) checking the compatibility of multiplexing, and (5) minimizing the number of primers/probes needed to achieve desired coverage by using set coverage analysis. . The key steps are shown in **Fig. 2** and described in more details in the following sections.

Collection of related sequences
        All available sequences for the targeted types, groups, or subtypes are collected either from the GenBank at NCBI or from the HIV Sequence Database and the Influenza Sequence Database, both at the Los Alamos National Laboratory.

Multiple sequence alignment and phylogenetic analysis
        All target sequences are aligned using ClustalW (*Chenna et al. 2003*) and distance trees are built using the Neighbor-Joining method. Additional epidemiological analyses for geographical and temporal clustering are also performed to identify prevalence strains and predict the future complexity of regional and global genetic diversity (*Gilbert et al. 2007; Munster et al. 2007*). The results from these analyses are used to guide the selection of target sequences.

Fig. 2. A semi-automated primer/probe design pipeline for identifying the minimal set of multiplexed degenerate non-overlapping universal tagged primers. The key steps in the pipeline are shown including primer/probe selection using *Primer3*, ranking all possible primer sets based on coverage, specificity, and finally selecting the mimimal multiplexed set using a greedy algorithm to identify the minimal, non-overlapping primer-probe set to detect all viral strains of a given type, group or subtype.

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Finding the consensus sequences for target types or subtypes

The consensus sequences are generated from the multiple sequence alignment for the target groups according to the majority rule and used in the primer and/probe design.

Identifying all possible primer/probe sets across the entire sequence space

Primer pairs and their corresponding probes are selected by *Primer3* (*Rozen & Skaletsky 2000*) according to the user's design requirements. The number of primer-probe sets picked by *Primer3* is equal to the length of the consensus sequence so that the primer/probe sets cover the entire sequence space. This is different from all published primer design methods that use only the conserved sequence regions in their primer design. Relying on the conserved regions often leads to high false positive rates against subtypes other than the targets and also faces difficulty in covering some unique strains. To further reduce the false positive rate, the 5' and 3' ends of conserved regions are excluded when designing subtype-specific signatures (*Hoffmann et al. 2001*). All internal gap regions within the multiple sequence alignment are also excluded by *Primer3*.

Selecting the best primer/probe sets

To improve the detection coverage, degenerate bases are introduced at different positions for each primer-probe set according to the frequency of the alternative bases as governed by a user-defined degeneracy cutoff. While degenerate primers are as easy and cheap to produce as regular unique primers and well suited for amplification of variant sequences, they introduce two new problems. First, the effective concentration of the desired primers is decreased by the presence of undesired primers. Second, the presence of the undesired primers can lead to erroneous amplification (*Souvenir et al. 2007*). Therefore, it is important to use primers of relative low degeneracy to realize the benefits of the degenerate primer while minimizing these undesired effects. So only a low level of degeneracy (typically less than 10 fold) is introduced for each primer or probe. The overall coverage of each degenerate primer-probe set is calculated against the target strains. Another unique feature of our primer design is the use of 5' universal tags to increase the amplification efficiency and homogeneity for the multiplexed amplification reaction. The universal tags (*e.g.,* M13 *forward* and *reverse* primers) are used as the amplification primers. After initial amplification through the target-specific primers during the very first few cycles, the universal tags take over as the amplification primers for subsequent amplification cycles. Compatibility of the universal tags with the target-specific primer pairs is analyzed using *mFold (Zuker 2003)*. Any primer-probe set that becomes incompatible (*e.g.,* homodimer, heterodimer or hairpin formation) after the addition of the 5' universal tags is discarded from further considerations. Robustness of each remaining primer-probe set is also checked using *Blastn* as well as *Thermonucleotide*Blast (http://public.lanl.gov/jgans/tntblast/) based on hybridization melting temperature against the human genome. Any primer-probe set with a high probability of amplifying non-target sequences is discarded. Finally, the remaining primer-probe sets are ranked according to both their coverage of the target sequences and their false positive rate against the non-target types or subtypes.

Identifying the minimal set of multiplexed primers/probes

Multiplex compatibility is a property of the primer/probe and the PCR conditions. Algorithmically, multiplex compatibility must satisfy three criteria: (1) different primer-probe

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sets must not bind to each other. If binding is more stable than a user-defined melting temperature threshold (T_m^{dimer}), the oligos are not compatible; (2) the range of melting temperatures for a set of multiplexed primers must be within user specified limits (ΔT_m); and (3) a maximum of *N* pairs (typically 5) of PCR primers can be combined into a single multiplex reaction. This last heuristic reflects a commonly accepted rule of thumb for performing multiplex PCR. DNA melting temperatures are calculated using the near-neighbor DNA hybridization parameters of SantaLucia (*SantaLucia 1998*). These parameters account for the contribution of both salt and oligo strand concentrations to duplex stability. When oligos contain degenerate bases, the concentration of any particular DNA molecule decreases. This decrease in concentration causes a reduction in melting temperature to its intended target, which is accounted for in our compatibility calculations.

The multiplexed primer set selection has been classified as an optimization problem for finding the minimal primer set to cover all targeted sequences (*Linhart & Shamir 2005*). Many greedy heuristic algorithms have been implemented to address this problem (*Doi & Imai 1997; Rose et al. 2003; Jarman 2004; Linhart & Shamir 2005; Rachlin et al. 2005; Jabado et al. 2006; Souvenir et al. 2007*). We have developed a similar greedy algorithm to identify the minimal set of multiplexed degenerate universal tagged primers and probes. Our algorithm proceeds as follows. First, the top-ranked primer-probe set with the highest coverage from the previous step is selected. The remaining primer/probe sets are re-ranked according to the coverage for the strains that are not covered by the first primer/probe set. The highest ranked primer-probe set is then checked for overlap with the first primer/probe set. If there is no overlap, it is then selected to check multiplexing by determining its compatibility with the first set. If not compatible, then the second highest ranked primer-probe set is checked until a compatible set is found. Using an iterative process, the minimal set of primers and probes that cover all intended target strains or meet the minimal coverage requirement is identified.

Results and Discussion

Using this semi-automated signature design pipeline, we have identified the minimal primer/probe sets for many different types and subtypes of HIV and influenza viruses. A typical minimal set consists of 3-4 multiplexed degenerate primers/probes and has 90-100% coverage of their target strains. They are currently being tested in the lab to screen a collection of clinical and field samples.

There are several advantages of using the minimal set of multiplexed degenerate universal tagged primers and probes: (1) degenerate primer-probe sets support detection of a diverse set of strains, including potentially new emerging strains; (2) multiplexing allows increased coverage while maintaining a relatively low level of degeneracy in a single primer-probe set, but without interference among different primer sets. Multiplexed assays also permit the detection of mixed virus infections that could be missed by a single real-time reaction (*Wu et al. 2008*); (3) addition of universal primer amplification primers can dramatically increase detection sensitivity, which is critical in detecting early infection where the viral load may be extremely low; (4) the use of non-overlapping primer-probe sets will avoid competition among different primer sets for the same target sequences and thus increase the number of available

targets, leading to higher sensitivity. Furthermore, the use of phylogenetic analysis to identify geographical, temporal or host-specific clustering as a part of our primer-probe design pipeline facilitates development of specific primer-probe sets for different geographic locations or hosts with increase specificity.

ACKNOWLEDGMENTS

References:

Auewarakul, P., K. Sangsiriwut, K. Chaichoune, A. Thitithanyanont, W. Wiriyarat, T. Songserm, R. Ponak-nguen, J. Prasertsopon, P. Pooruk, P. Sawanpanyalert, P. Ratanakorn and P. Puthavathana (2007). "Surveillance for reassortant virus by multiplex reverse transcription-PCR specific for eight genomic segments of avian influenza A H5N1 viruses." J Clin Microbiol **45**(5): 1637-9.

Chen, Z. and P. G. Plagemann (1995). "Detection of related positive-strand RNA virus genomes by reverse transcription/polymerase chain reaction using degenerate primers for common replicase sequences." Virus Res **39**(2-3): 365-75.

Chenna, R., H. Sugawara, T. Koike, R. Lopez, T. J. Gibson, D. G. Higgins and J. D. Thompson (2003). "Multiple sequence alignment with the Clustal series of programs." Nucleic Acids Res **31**(13): 3497-500.

Coleman, J. W., E. Ogin-Wilson, J. E. Johnson, F. Nasar, T. P. Zamb, D. K. Clarke, R. M. Hendry and S. A. Udem (2007). "Quantitative multiplex assay for simultaneous detection of the Indiana serotype of vesicular stomatitis virus and HIV gag." J Virol Methods **143**(1): 55-64.

Constantine, N. and R. Zhao (2005). "Molecular-based laboratory testing and monitoring for human immunodeficiency virus infections." Clin Lab Sci **18**(4): 263-70.

Constantine, N. T. and H. Zink (2005). "HIV testing technologies after two decades of evolution." Indian J Med Res **121**(4): 519-38.

Daum, L. T., L. C. Canas, C. A. Schadler, V. A. Ujimori, W. B. Huff, W. J. Barnes and K. L. Lohman (2002). "A rapid, single-step multiplex reverse transcription-PCR assay for the detection of human H1N1, H3N2, and B influenza viruses." J Clin Virol **25**(3): 345-50.

Doi, K. and H. Imai (1997). "Greedy Algorithms for Finding a Small Set of Primers Satisfying Cover and Length Resolution Conditions in PCR Experiments." Genome Inform Ser Workshop Genome Inform **8**: 43-52.

Ehlers, B., K. Borchers, C. Grund, K. Frolich, H. Ludwig and H. J. Buhk (1999). "Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers." Virus Genes **18**(3): 211-20.

Gibellini, D., F. Gardini, F. Vitone, P. Schiavone, G. Furlini and M. C. Re (2006). "Simultaneous detection of HCV and HIV-1 by SYBR Green real time multiplex RT-PCR technique in plasma samples." Mol Cell Probes **20**(3-4): 223-9.

Gilbert, M. T., A. Rambaut, G. Wlasiuk, T. J. Spira, A. E. Pitchenik and M. Worobey (2007). "The emergence of HIV/AIDS in the Americas and beyond." Proc Natl Acad Sci U S A **104**(47): 18566-70.

Hoffmann, E., J. Stech, Y. Guan, R. G. Webster and D. R. Perez (2001). "Universal primer set for the full-length amplification of all influenza A viruses." Arch Virol **146**(12): 2275-89.

Jabado, O. J., G. Palacios, V. Kapoor, J. Hui, N. Renwick, J. Zhai, T. Briese and W. I. Lipkin (2006). "Greene SCPrimer: a rapid comprehensive tool for designing degenerate primers from multiple sequence alignments." Nucleic Acids Res **34**(22): 6605-11.

Jarman, S. N. (2004). "Amplicon: software for designing PCR primers on aligned DNA sequences." Bioinformatics **20**(10): 1644-5.

Linhart, C. and R. Shamir (2005). "The degenerate primer design problem: theory and applications." J Comput Biol **12**(4): 431-56.

Mansky, L. M. (1998). "Retrovirus mutation rates and their role in genetic variation." J Gen Virol **79 (Pt 6)**: 1337-45.

Munster, V. J., C. Baas, P. Lexmond, J. Waldenstrom, A. Wallensten, T. Fransson, G. F. Rimmelzwaan, W. E. Beyer, M. Schutten, B. Olsen, A. D. Osterhaus and R. A. Fouchier (2007). "Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds." PLoS Pathog **3**(5): e61.

Rachlin, J., C. Ding, C. Cantor and S. Kasif (2005). "MuPlex: multi-objective multiplex PCR assay design." Nucleic Acids Res **33**(Web Server issue): W544-7.

Rose, T. M., J. G. Henikoff and S. Henikoff (2003). "CODEHOP (COnsensus-DEgenerate Hybrid Oligonucleotide Primer) PCR primer design." Nucleic Acids Res **31**(13): 3763-6.

Rozen, S. and H. Skaletsky (2000). "Primer3 on the WWW for general users and for biologist programmers." Methods Mol Biol **132**: 365-86.

SantaLucia, J., Jr. (1998). "A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics." Proc Natl Acad Sci U S A **95**(4): 1460-5.

Souvenir, R., J. Buhler, G. Stormo and W. Zhang (2007). "An iterative method for selecting degenerate multiplex PCR primers." Methods Mol Biol **402**: 245-68.

Steinhauer, D. A. and J. J. Holland (1987). "Rapid evolution of RNA viruses." Annu Rev Microbiol **41**: 409-33.

Wu, C., X. Cheng, J. He, X. Lv, J. Wang, R. Deng, Q. Long and X. Wang (2008). "A multiplex real-time RT-PCR for detection and identification of influenza virus types A and B and subtypes H5 and N1." J Virol Methods **148**(1-2): 81-8.

Zuker, M. (2003). "Mfold web server for nucleic acid folding and hybridization prediction." Nucleic Acids Res **31**(13): 3406-15.

LIMS Evaluation & Recommendation
for High-Throughput Flu Lab

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Purpose

The purpose of this document is to summarize LANL's LIMS (Laboratory Information Management System) evaluation and down-selection process. The document concludes with a LIMS product recommendation for the HT Flu Laboratory Project Management Office (PMO).

Background

After composing a detailed statement of needs for the HT Flu Laboratory LIMS (Laboratory Information System), a call for proposals (RFP) was sent out to a total of six leading LIMS companies on September 27, 2007. The list of targeted companies included StarLIMS, LabWare, LabVantage, BlazeLIMS, LabLynx, and ThermoFisher. The deadline for submitting bids to LANL was set to October 18, 2007. By that deadline, LANL had received a total of five bids. The only company that decided not to submit to our RFP was ThermoFisher. The main reason for not submitting was that ThermoFisher's Nautilus LIMS turned out to be incompatible with the HT Flu Lab's already existing 64-bit Windows Server and SQL Server database environment. After receiving the bids, a team-based review and ranking process of all the submissions took place. LANL's team of reviewers included Craig Blackhart, Maura Wilhelm, Sandra Cruz, and Torsten Staab.

Evaluation Criteria and Results

At the beginning of the evaluation process, each reviewer was given a list of evaluation criteria to be used during the scoring process. The list of evaluation criteria included:

- Cost for base system
- Cost for licensing
- Requirements coverage right out-of-box
- Customizability
- Support Cost
- Availability of a software development kit or API (Application Programming Interface)
- Design and ease of use of the LIMS's user interface
- Support of standard (3rd Party) software
- Scalability (e.g., multi-sites, number of users)
- Webservices support
- Web-based front end
- General IT (state-of-the-art)
- IT architecture
- Security
- Integration capabilities
- Logistics support
- Data management

- Auditing
- Standard compliance (e.g., HIPPA, CFR 21 Part 11)

LIMS Product Ranking

Rank	LIMS Product	Number of Votes (Maximum: 4 out of 4)
1st	StarLIMS	4
2nd	LabVantage LabLynx	2 2
3rd	LabWare	1
4th	BlazeLIMS	0

Reviewer Comments by Product

LIMS Product	Comments
StarLIMS	Pros: excellent out-of-box coverage of our requirements (100%), nice user interface, scalability, traceability, lab logistics support, IT architecture, integration capabilities, user interface design, , very impressive customer base (e.g., CDC, DOD, DHS, USDA, DOJ, including all major pharmas & biotechs across the globe; see pages 16-22 of their response for a partial list of customers), HIPPA-, 21 CFR Part 11, and ISO 17025 compliant StarLIMS is the only ISO 9000-certified LIMS company; it conducts LIMS development and implementation projects in accordance with Project Management Institute (PMI) guidelines (->high quality project management and customer relationship management) Cons: a little pricey, but reasonable given its feature set and product quality ($9K per full user license, $5K for limited user license)
LabVantage	Pros: very good out-of-box coverage of our requirements (~98%), decent user interface Cons: limited configurability, not HIPPA compliant, very limited user activity tracking capability, too many modules, high training requirements, pricey
LabLynx	Pros: covers about 80% of our requirements out-of-box, provides a LIMS hosting option, very extensive sample and data tracking capabilities, submitted a very detailed response, offers attractive unlimited external user option via a one-time $15K WebAccess application purchase Cons: limited reporting capability, primitive user interface, limited configurability, only covers about 40% of what we need out-of-the-box
LabWare	Pros: very modular, decent out-of-box coverage of our

	requirements (~90%), integration, configurability
	Cons: RFP response was written very vague, pricey procurement and support
BlazeLIMS	Pros: inexpensive (due to lack of functionality)
	Cons: requires client-side software installation (->system maintenance nightmare), no develop kit or API, covers only about 2% of what we need out-of-box, did not spend sufficient time on their response (i.e., very superficial, low quality, ignored most of our requirements)

Product Cost

The following table summarizes the procurement cost of each product. Please note the comments in the cost column, as they explain the major cost difference between the products. While trying to compare cost, please also keep in mind each product's out-of-box coverage of our requirements. On paper, for example, BlazeLIMS appears to be the cheapest product. However, this product only covers about 2% of what we actually would need. It could easily cost more than $750K to get the product's functionality to where we would need it to be. The extra time, cost, and implementation risk automatically disqualifies products such as BlazeLIMS.

LIMS Product	Cost
StarLIMS	$252K
	(= $99,750 total license cost + $144,115 for training and professional service costs (includes 68 days of off-site + 29 days of on-site customization) + $7,500 travel and lodging budget for on-site visits and admin/user training @ StarLIMS headquarters in Hollywood, Florida)
	Note: $17,700 (which amounts to be 14% of the total license cost) for annual software maintenance for year 2 is not included in total above; first year of software maintenance is "free."
LabVantage	$309K
	(=89.5K for software and licenses + 125 days of customization + user training)
LabWare	$180K
	(includes 55 days of customization)
LabLynx	$48.5K
	(includes 10 days of customization + user training)
BlazeLIMS	$35K
	(does not include any customization)

Note that in the LIMS industry, annual software maintenance fees of 12-16% of the initial procurement cost (excluding any customization costs, of course) are quite customary. The

costs listed in the table above include the year-one licensing fees for up to five internal and five external LIMS users. Additional user seats can be purchased at any time.

Recommendation

After a thorough evaluation of all five bids, the reviewers unanimously concluded that StarLIMS provided the best overall solution for our project. Considering the product's out-of-the-box functionality, its scalability, 3rd party integration capabilities, extensibility, level of maturity, quality, and industry reputation, StarLIMS emerged as the clear winner in this competition. The product (in various incarnations) has been on the market for almost 20 years and they have hundreds of installations worldwide. Besides the formal evaluation process outlined above, LANL review team members also participated in StarLIMS product demos at LabAutomation'07 and on site at LANL in August 2007.

Furthermore, the LANL review team lead also checked references of current StarLIMS customers, such as the National Bioforensic Analysis Center (NBFAC) in Frederick, MD. We also consulted with Dr. Robert D. McDowall (Kent, UK), who is an internationally renowned LIMS expert. The feedback we received in both cases on StarLIMS was very positive.

From the Stock Market, Nov 13, 2007: *StarLIMS announces Third Quarter 2007 Financial Results* (Source: http://ir.starlims.com/releasedetail.cfm?ReleaseID=275466)

STARLIMS Technologies Ltd. (Nasdaq:LIMS), a leading provider of laboratory information management systems (LIMS), today announced that revenues for the third quarter of 2007 were $6.2 million, a 46.3% increase over revenues of $4.2 million reported for the same period in 2006. Product revenues for the period were $3.6 million, up 73.0% from $2.1 million reported for the third quarter of 2006. Services revenues for the period were $2.5 million, up 19.7% from $2.1 million reported for the third quarter of 2006.

Revenues for the first nine months of 2007 were $17.1 million, up 34.2% from $12.7 million for the first nine months of 2006. Product revenues for the first nine months of 2007 were $9.9 million, a 43.7% increase over the comparable period in 2006. Services revenues for the first nine months of 2007 were $7.2 million, up 23.2% from $5.9 million for the first nine months of 2006.

STARLIMS's business model is characterized by relatively large transactions, with a year-to-date average selling price on direct sales of approximately $600,000. Our management believes that a comparison of STARLIMS's results in a longer term perspective provides better insight into the underlying growth of our business.

"Our growth, driven by our STARLIMS V10, continues to outpace the overall growth of the LIMS market. STARLIMS V10 is the only web-based LIMS product designed to consolidate disparate business processes into one platform and allow for data sharing within the laboratory and across the enterprise," said Itschak Friedman, CEO of STARLIMS. "The capabilities of our STARLIMS V10 platform also allow us to expand our market opportunities by entering adjacent markets such as the clinical laboratory information systems or LIS market." …

Cash, cash equivalents and marketable securities amounted to $33.9 million on September 30, 2007, compared to $6.5 million on December 31, 2006. The increase in cash was primarily due to our initial public offering in the United States on May 23, 2007.

Next Steps

Assuming approval from the HT Flu Lab project management team to proceed with StarLIMS to the next stage, the team proposes to jointly develop a phased LIMS implementation plan with StarLIMS personnel. We currently anticipate completion of the phased implementation plan by the end of December 2007. This milestone then will be followed by an official LIMS project kick-off meeting at LANL at the beginning of January 2008. Pages 23-39 of the attached StarLIMS RFP response provide a very detailed description of the StarLIMS recommended implementation process.

Appendix

This appendix summarizes the HT Flu Lab LIMS requirements that were given to each LIMS vendor to prepare their bids.

A. General IT Requirements

A1. Windows Server Support
The LIMS shall be able to run on DELL-based, multi-core server hardware that uses the 64-bit edition of Microsoft Windows Server 2003 R2 as operating system.

A2. Microsoft SQL Server Support

The LIMS shall support the 64-bit edition of Microsoft SQL Server 2005 as main data storage back end.

A3. Number of anticipated LIMS Users

We are planning a phased LIMS roll out with respect to functionality and number of users.

Year 1
3-4 external users (i.e., sample pre-loggers, result requesters), with a maximum of two concurrent external users at any time.
3-4 internal users, with a maximum of two concurrent internal users at any time.

Year 2
5-8 external users, with a maximum of three concurrent external users at any time.
3-4 internal users, with a maximum of two concurrent internal users at any time.

Year 3
9-15 external users with a maximum of five concurrent external users at any time.
5-6 internal users, with a maximum of three concurrent internal users at any time.

B. Architectural Requirements

B1. Multi-Tier Design
The LIMS architecture shall incorporate load balancing, redundancy, automated failover, clustering and expansion of data storage.

B2. Multi-Site Support

The LIMS architecture shall allow for seamless integration and (remote) management of multiple, heterogeneous laboratory sites. Multi-site administration features for remote management of users, rapid deployment of new workflows, methods, and forms across multiple sites, and sharing/exchange of data shall be provided.

B3. Web-based User Interface

The LIMS shall provide a zero-install, computing platform-independent, web browser-based user interface. At a minimum, the system should support the latest versions of Internet Explorer on Windows and Macintosh platforms.

C. Security Requirements

C1. Role-based, Multi-Level Security Model

C1.1. The LIMS shall support a role-based, need-to-know type access control system for user authentication. A LIMS user should be able to occupy multiple roles concurrently.

C1.2. Furthermore, the system shall provide a custom role definition feature and allow for grouping of users.

C1.3. The LIMS administrator shall be able to grant and revoke document and form access privileges at the individual user level in real-time.

C2. Encrypted Data Exchange

The LIMS shall support a standardized, encrypted communication protocol such as SSL (Secure Socket Layer) between the browser-based client and the server application.

C3. Security/Audit Trailing

C3.1. The LIMS shall have the ability to configure group and individual security settings.

C3.2. The LIMS shall control access by internal users and external client user groups.

C3.3. The LIMS shall control access control by project and workflow procedure.

C3.4. The LIMS shall track all user activities, such as data entry, data uploads/downloads, data edits, data deletions, record views, and data requests automatically.

C3.4.1 The LIMS shall control access to the audit trail information in a secure fashion.

C3.5. The LIMS administrator shall be able to search the secure log file using a variety of parameters (e.g., search by user, date, time, site, location, instrument, document, method, or file).

D. Integration Requirements

D1. Web Services Support

The LIMS should provide web services-based application programming interface that support programmatic data import, export, and result queries.

D2. Third Party Integration

D2.1. The LIMS shall integrate with third party software and hardware. For example, the LIMS shall be able to communicate bi-directionally with laboratory instrumentation to start, pause, and stop laboratory processes, upload/download data from/to instruments, and to obtain instrument-level status information.

D2.2. The LIMS shall be able to upload and link pertinent e-mails and other documents to associated samples.

D3. Developer Tools

D3.1. The LIMS shall provide developer user utilities to create custom configuration of application screens, data fields, and data analysis.

D3.2. The LIMS shall provide for configuration management (i.e., versioning) to ensure that only one version (e.g., a workflow, user interface screen, or report) is used across all nodes.

D4. Barcoding Support

D4.1. The LIMS shall support the most commonly used 1D and 2D barcode formats.

D4.2. The LIMS shall be able to read in data from standard handheld and stationary barcode readers.

D4.3. The LIMS shall be able to print out sample and container barcode labels in various formats using standard barcode printers from companies such as Zebra and Symbol.

E. Logistical Requirements

E1. Workflow Centric

The LIMS shall support dynamic workflow requirements.

E2. Workflow and Method Editor

The LIMS shall provide tools (e.g., a graphical editor or wizard) that enable the administrator to create new and/or modify existing work flows and laboratory methods.

E3. Projects / Work Types

E3.1. The LIMS shall have the ability to organize work into project types (e.g., Sequencing, Culturing, Screening, Repository) for configuration, processing, and access control.

E3.2. The LIMS shall be able to transfer an item along with all its associated data within the system from one project to another.

E3.3. The LIMS shall be able to link individual samples and/or sample batches (i.e., groups of samples) to a case record. A case record, for example, could be a patient and/or animal data record. Case record information would be entered by the user during the sample pre-log and/or log-in screens.

E4. Shipments

E4.1. The LIMS shall be able to track incoming and outgoing package shipments. Basic information would include: a) names and associated contact information of sample/record sender and receivers, b) date/time stamps of events, c) record ship to and sent to, d) shipment tracking number, and e) optional shipping comments.

E4.2. The LIMS shall be able to attach scanned shipping documents (e.g., shipment manifests, packing slips, and courier receipts) to the shipment data records.

E4.3. The LIMS shall provide a utility to generate groups with items for an outgoing shipment.

E4.4. The LIMS shall calculate the depletion of item stock amounts when shipped outside of the storage facility.

E5. Sample Accessioning

E5.1. The LIMS shall assign unique, alpha-numerical case numbers to new records. Case numbers shall not be recycled/re-used after decommissioning of case records.

E5.2. The LIMS shall have the ability to maintain unique records for each item (accession item) received in a case (i.e., batch or receipt group). The Accession item ID should be composed of the case number plus a running number postfix (e.g., "abc12320070830.01", "abc12320070830.02")

E5.3. The LIMS shall provide a feature that allows generating individual accession item IDs as well as groups of accession item IDs.

E5.4. Provide an accession import utility that would fill in accession item information from a digital file.

E5.5. The LIMS shall also maintain the original item ID along with the accession item ID (i.e., e.g., ID of the mother plate or tube).

E5.6. The LIMS shall record the amount and amount unit for each accessioned item.

E5.7. The LIMS shall record the storage location of each accessioned item (e.g., site, building, room, freezer unit ID, shelf ID, box ID).

E6. Sample Processing

E6.1. The LIMS shall provide a feature to create multiple samples at a time.

E6.2. The LIMS shall provide a feature to print individual as well as batch sample labels.

E6.3. The LIMS shall be able to import and store ASCII (e.g., XML) and binary (e.g., JPG, AVI) data streams at the case and individual sample levels.

E6.4. The LIMS shall provide a feature to create and uniquely identify portion/sup portion of samples (e.g., dilutions, cultures/sub-cultures plating, sample alteration, sub-aliquots).

E6.5. The LIMS shall establish a unique identifier to relate data to accession and testing samples.

E6.6. The LIMS shall create and track process controls, reagents, and instruments used during sample processing.

E6.7. The LIMS shall be configurable to generate alerts when the number of items in a particular group falls below a certain level (e.g., we create 10 aliquots of an item; after 5 aliquots have been distributed/consumed, generate an alert to the inventory manager).

E6.8. The LIMS shall track the consumption of sample materials, reagents, and labware consumables during sample processing.

E6.9. The LIMS shall provide a means to attach comments to each sample.

E7. Workflow/Business Rules

E7.1. To expedite the data entry process, the LIMS shall provide default values for most commonly used data fields and provide pre-defined but re-configurable data selection lists (e.g., drop down boxes, check boxes).

E7.2. The LIMS shall provide the ability to deviate from standard workflows given proper authorities, but require the user to document the deviation reasons.

E7.3. The LIMS shall provide a feature to monitor sample progress according to selected testing plan(s).

E7.4. The LIMS shall have the ability to automated procedures and/or workflows based on positive / negative or specific result values.

E7.5. The LIMS shall be able to manage QC/QC review and approval cycles (e.g., alert the appropriate personnel to review and approve pending results). Lab-specific business rules shall determine which electronic signatures are required before final release.

E7.6. During the release process, all information related to the reviewed sample(s) shall be easily accessible (i.e., instrument maintenance records, analyst's training records, record pertaining to standards that were used, and audit trail records and signatures gathered at all the previous workflow steps).

E7.7. The LIMS shall support sample and/or batch (re-)prioritization.

E8. Equipment, Storage Locations, and Supply Maintenance

E8.1. The LIMS shall store information about laboratory equipment (e.g., manufacturer, model, serial number, supplier, procurement date, procurement cost, user manuals and software versions, service, and calibration records, owner, location, and internal inventory number).

E8.2. The LIMS shall generate automated reminder for upcoming service dates, calibration dates, and renewals.

E8.3. The LIMS shall track supply information including order/receipt information, shipment tracking information, receive date, ordered by, order date, supplier, manufacturer, supply formulations, lot number, expiration date, scanned MSDS data sheets, internal owner, storage location, re-order threshold (e.g., number of units lefts, min. stock weight / volume with unit).

E8.4. The LIMS shall provide the ability to easily link equipment, reagents, and other supplies to test methods and samples during processing.

E8.5. The LIMS shall provide the user with acceptability alerts when equipment or supply is expired, low stock, out of service, or out of calibration.

E8.6. Storage locations shall be managed hierarchically (e.g., site->building->room ->storage unit | [device [-> shelf | table [->box | container [-> plate| tube]]]).

E8.7. The LIMS should be able to flag and/or lock-out data from expired or out of calibration equipment. The data may only be reported with laboratory manager and/or QA approval and justification.

E9. Training Management

E9.1.   The LIMS shall manage the training records of laboratory personnel.

E9.1.1. Provide a user interface for creating, modifying, and deleting training plans and classes.

E9.1.2. Enable the training administrator and workflow manager to associate mandatory and optional training plans/classes to laboratory processes.

E9.1.3. Allow for upload and storage of digitized training certificates.

E9.1.4. Track training expiration dates and send out automatic training reminders.

E9.1.5. Provide a centralized area from which training materials can be downloaded.

E9.1.6. Check training records before allowing a user to initiate a certain laboratory process (e.g., test procedure) to ensure that (s)he has had the proper training and is current on his/her required training.

F. Data Management Requirements

F1. Sample Pre-Log

The LIMS shall provide a web-based sample pre-log feature, enabling remote and local users to pre-register samples, obtain unique sample identifiers, print barcodes, and upload sample-related multi-media files (i.e., one or more image, text, or voice files per sample). Information submitted via the pre-log screen(s) would encompass time and data of sample pre-log, name and contact information of sample owner/requester, number of samples, type(s) of samples, format of sample(s), quantity (e.g., weight, volume) of sample, shipping container type(s), customer's own sample reference/ID numbers, analysis check list, required turnaround time, and barcode (1D and/or 2D) printing/download.

F2. Sample Log-in

The LIMS shall provide a web-based sample log-in feature that enables local (and remote) LIMS users to log-in samples. Similar to R13, this feature shall also enable the user to obtain unique sample identifiers, print barcodes, and upload sample-related multi-media files. The sample log-in screen(s) will also contain data fields to track sample chain-of-custody related information and comments (e.g., written notes about state of received sample container).

F3. Result Query

F3.1. LIMS shall provide a web-based result data query engine that enables external and internal LIMS users to query result data sets using a variety of search criteria (e.g., search by submitter name, time, date, sample type, site, location, instrument, and laboratory technician).

F3.2. Besides web-based data viewing, the user shall also have the ability to download sample and result-related data files based.

F3.3. The LIMS shall manage access to result data according to assigned roles and ownerships to ensure data confidentiality and security.

F3.4. The LIMS shall provide an API (Application Programming Interface; preferably .NET or Web Services-based) to allow for programmatic result data access and data exchange in general.

F4. Test Result Management

F4.1. The LIMS shall provide a result data import feature to minimize manual data entry and human error. Data import from the following instruments shall be provided:
a) ABI 7900 Sequencer (first year; additional devices expected in years 2 and 3)

F4.2. The LIMS shall be able to record multiple types of test result data per sample. Possible result formats are:
a) numeric and alpha-numeric data

b) text files (e.g., CVS, plain ASCII)

> c) uncompressed digital images
>
> d) native analysis and binary files

F4.3. The LIMS shall be able to record multiple results by sample and by test. That is, if a test was repeated or analyzed more than once, mark and report a primary result, and/or report multiple results with comments.

F4.4. The LIMS shall have the ability to search and compare test result data (e.g., search by batch ID, sample ID, accessioning ID, technician, submitter, location, instrument, date (and time)).

F4.5. The LIMS shall provide a tool to summarize/consolidate test results belonging to an individual sample or sample batch for review.

F5. Automatic Data Capture

F5.1. The LIMS shall be able to directly import result data from laboratory instrumentation in an on-demand and/or scheduled fashion.

F5.2. Imported instrument data shall be automatically linked to the corresponding test method and sample(s).

F6. Manual Data Capture

F6.1. The LIMS shall provide a user interface that allows for manual uploading of result data files and manual data entry.

F6.2. The LIMS shall provide means for attaching comments to samples and results.

F7. CRM (Customer Relationship Management)

F7.1.   The LIMS shall provide basic CRM functionality.

F7.1.1. Screens to create, edit, search, and delete customer contact information.

F7.1.2. Profile screens (e.g., to generate order history, pre-logged samples, logged samples, results).

F8. Report Generation

F8.1. The LIMS shall provide a custom report generator and be able to output reports in standard file formats, such as XML, HTML, PDF, ASCII, and EXCEL.

F8.2. The LIMS shall be able to capture all comments, mark which ones to send to client, and allow editing of comments with internal traceability.

F8.3. The LIMS shall be provide means for reporting partial results.

F8.4. The LIMS shall flag results that are being reported without complete review and approval.

F9. Data Integrity

The LIMS shall maintain data accurately and preserve information integrity.

G. Auditing/Tracking Requirements

G1. Chain of Custody (COC)

G1.1. The LIMS shall update the COC record for a group of samples or items, indicating the person sending or receiving the sample(s), the action date, the shipment date, process status, and storage location.

G1.2. The LIMS shall provide a summary screen displaying the COC history of a sample batch and/or individual sample.

G1.3. Sample batches could be distributed among various sites / storage locations. The LIMS shall track the location(s) of sample batches and individual samples (e.g., site, building, room, storage unit, device, shelf, table, box, container, plate, tube). Also see E8.6.

G1.4. The LIMS shall provide a feature to create ad-hoc sample groups/batches and document their transfer between internal sites/departments.

G1.5. The LIMS shall provide a comment field with each sample and/or batch.

G1.6. The LIMS shall track, associate, and document all pertinent data pertaining to reagents, standards, and instrumentation used on each sample. For example, vendor, date opened, expiration date, lot number, and analysts involved in sample preparation and/or analysis.

H. Standard Compliance Requirements

H1. HIPPA and CFR 21 Part 11 Compliant

The LIMS must be HIPPA and CFR 21 Part 11-compliant with respect to its electronic document management, handling of digital signatures, and audit trailing.

RFP: Laboratory Information Management System (LIMS)	Scoresheet							
RFP No.: Los Alamos Statement of Needs, dated 9/27/2007	**Evaluator Comments:** Note any major benefits or deficiencies in a proposal or differentiating factors that led evaluator to award very high or low scores should listed below.						**Evaluator Comments**	
Evaluators: Torsten Staab, PhD; Craig Blackhart; Sandra Cruz; Maura Wilhelm	**Minimum Score Requirement:** Proposals which do not score at least 70% of total possible quality points for all evaluation categories will be disqualified and will not be considered for award.						I put percentages in each box, but for the total score section I calculated a score based the sum of the percentage * weight for the section.	

The more detailed the response, the more accurately it could be graded.

SCORE		GUIDELINE	
Excellent = 90 - 100%		Exceptional response, exceeded criteria	0's given for no response
Good = 81 - 90%		Competently met criteria with no deficiencies	1's given for yes/no response or response that did not answer request
Average = 61 - 80%		Competently met criteria with minimal deficients	
Below Average = 41 - 60%		Met criteria but with significant deficiencies	
Poor = 1 - 40%		Minimally met criteria	
No Response = 0%		Did not respond to criteria	

CRITERIA	TOTAL WEIGHT	BlazeLIMS	LabLynx	LabWare	LabVantage	StarLIMS	ThermoFisher	COMMENT
Section A General IT Requirements	Points	%	%	%	%	%	%	
A.1 Windows Server Support	200	0	75	75	75	75	75	100 for x64 support, 75 for x32 support
A.2 Microsoft SQL Server Support	200	0	50	50	1	100	0	0 for no support, 1 if not sure if it works or not
A.3 Number of anticipated LIMS Users	200	100	100	100	100	100	100	Every LIMS can support the number of licenses needed
Total Section A Score	**600**	**200**	**450**	**450**	**352**	**550**	**350**	
Section B Architechtual Requirements	WEIGHT	%	%	%	%	%	%	
B.1 Multi-Tier Design	100	0	79	79	94	99	79	
B.2 Multi-Site Support	100	1	1	1	89	99	49	
B.3 Web-based User Interface	500	1	95	95	95	95	1	1 if client install required at all
Total Section B Score	**700**	**6**	**555**	**555**	**658**	**673**	**133**	
Section C Security Requirements	WEIGHT	%	%	%	%	%	%	
C.1 Role-based, Multi-Level Security Model	50	0	81	1	92	99	81	
C.2 Encrypted Data Exchange	50	79	79	79	94	99	79	
C.3 Security / Audit Trailing	50	0	79	49	86	94	1	
Total Section C Score	**150**	**39.5**	**119.5**	**64.5**	**136**	**146**	**80.5**	
Section D Integration Requirements	WEIGHT	%	%	%	%	%	%	
D.1 Web Services Support	100	0	79	1	91	91	59	
D.2 Third Party Integration	100	29	59	1	86	94	69	
D.3 Developer Tools	200	19	1	1	56	96	79	
D.4 Barcoding Support	40	0	79	1	91	99	1	
Total Section D Score	**440**	**67**	**171.6**	**4.4**	**325.4**	**416.6**	**286.4**	
Section E Logistical Requirements	WEIGHT	%	%	%	%	%	%	
E.1 Workflow Centric	50	0	79	1	89	96	79	
E.2 Workflow and Method Editor	50	29	0	1	89	99	89	
E.3 Projects / Work Types	50	0	59	1	91	94	33	
E.4 Shipments	40	0	79	1	89	94	49	
E.5 Sample Accessioning	50	0	79	1	94	94	79	
E.6 Sample Processing	50	0	49	1	89	99	49	
E.7 Workflow / Business Rules	50	1	79	1	91	94	79	
E.8 Equipment, Storage Locations, and Supply	50	0	49	1	69	92	79	
E.9 Training Management	40	9	29	1	89	92	9	
Total Section E Score	**430**	**18.6**	**240.2**	**4.3**	**377.2**	**408.4**	**266.7**	
Section F Data Management Requirements	WEIGHT	%	%	%	%	%	%	
F.1 Sample Pre-Log	50	0	69	1	94	94	69	
F.2 Sample Log-In	50	0	79	1	91	96	69	
F.3 Result Query	50	0	9	1	76	94	69	
F.4 Test Result Management	50	0	29	1	89	94	89	
F.5 Automatic Data Capture	100	0	29	1	89	94	89	
F.6 Manual Data Capture	50	0	79	1	94	99	89	
F.7 CRM (Customer Relationship Management)	40	0	69	1	49	96	89	
F.8 Report Generation	50	0	19	19	59	94	81	
F.9 Data Integrity	50	0	69	1	89	91	1	
Total Section F Score	**490**	**0**	**233.1**	**13.9**	**404.6**	**463.4**	**358.1**	
Section G Auditing / Tracking Requirements	WEIGHT	%	%	%	%	%	%	
G.1 Chain of Custody (COC)	200	0	79	1	84	99	81	

Total Section G Score	200	0	158	2	168	198	162	
Section H Standard Compliance Requirements	**WEIGHT**	**%**	**%**	**%**	**%**	**%**	**%**	
H.1 HIPAA and CFR 21 Part 11 Compliant	200	0	100	100	50	100	100	
Total Section H Score	200	0	200	200	100	200	200	
Bidder's Total Points	3210	331.1	2127.4	1294.1	2521.2	3055.4	1836.7	StarLIMS has by far the most impressive and intuitive user interface, Thermo second, and the others have bad to really bad interfaces
Evaluation Basis (Points)		3210	3210	3210	3210	3210	3210	Overall, StarLIMS and LabVantage have the most functional LIMS and meet the most requirements out of the box
Evaluation Basis (%)		10%	66%	40%	79%	95%	57%	
Minimun Requirement (%)		70%	70%	70%	70%	70%	70%	
Cost Proposal					$ 149,880	$ 139,740		
Cost per Quality Point					$ 59	$ 46		

High-Throughput Laboratory Network (HTLN)

General IT Requirements for HTLN Instrumentation

Version: 1.1

Author: Torsten Staab, PhD, Los Alamos National Laboratory

1/23/2008

Purpose

The purpose of this document is to briefly outline the general IT requirements for automated laboratory instrumentation for UCLA's High-Throughput Influenza Laboratory (HTLN).

Objective

From an IT and systems integration perspective, the HTLN will be based on a Service-Oriented Architecture (SOA) approach. With respect to laboratory automation hardware and software, this means that every automated system (e.g., sample accessioning, sample storage and retrieval, culturing, sequencing, and data analysis; Figure 1) will be treated as a service provider.

Implementation

<u>Web services</u>

Rather than providing low-level , platform and programming language-dependent control interfaces for individual devices (e.g., an ActiveX control for each device), HTLN automation software and hardware suppliers are expected to provide macro-level (i.e., work cell-level), Web service-based control interfaces (Figure 1). Functional and logical grouping and abstraction of instrumentation in form of Web services will allow us to treat any sub-system/work cell as a black box with well-defined inputs and outputs for material and data.

By using a Web services-based approach to instrument control and communications, we not only provide a solid foundation for true plug-and-play integration of multi-vendor systems, but we also provide vendors with absolute implementation freedom.

LANL intends to provide the selected automation vendor(s) with the necessary WSDL-compliant (Web Service Description Language) interface definitions. It is important to note that these Web service definitions will be aimed at macro-level (i.e., work cell-level) controls rather than low-level controls. For example, a use case analysis of an automated sample storage and retrieval system would yield that such a system basically provides two services—1) Sample Check In, and 2) Sample Check Out. Internally, however, each of these services may require a certain sequence of steps, such as moving a robotic arm, scanning barcodes, etc. Rather than exposing low-level commands as Web services and requiring the service request to follow a certain device-specific call sequence, providing a macro-level command (i.e., service) such as "CheckIN <plate id> <location>" is much preferred.

For automated systems and/or system components that do not natively provide a Web service-based interface, but which are considered to be a service provider, the vendor(s) would be expected to provide a Web service-based "wrapper" around their legacy control interface(s).

Instrument Scheduling & Resource Management

As illustrated in Figure 1 and Figure 2, each work cell will have its own, dedicated, networked front end controller. Each work cell's front end controller is expected to provide and implement a certain set of Web services (based upon a prior work cell-level use case analysis). As mentioned earlier, LANL will provide the equipment vendor with the necessary WSDL interface definitions (and associated XML-based data structures).

While resource sharing across work cells may become necessary, it is important to note that for simplicity reasons each device or subsystem will be managed by only one dedicated, networked, controller. For example, to obtain access to a resource that is part of another work cell, a work cell controller would have to send a resource allocation request to the resource's local controller via its published Web service interface.
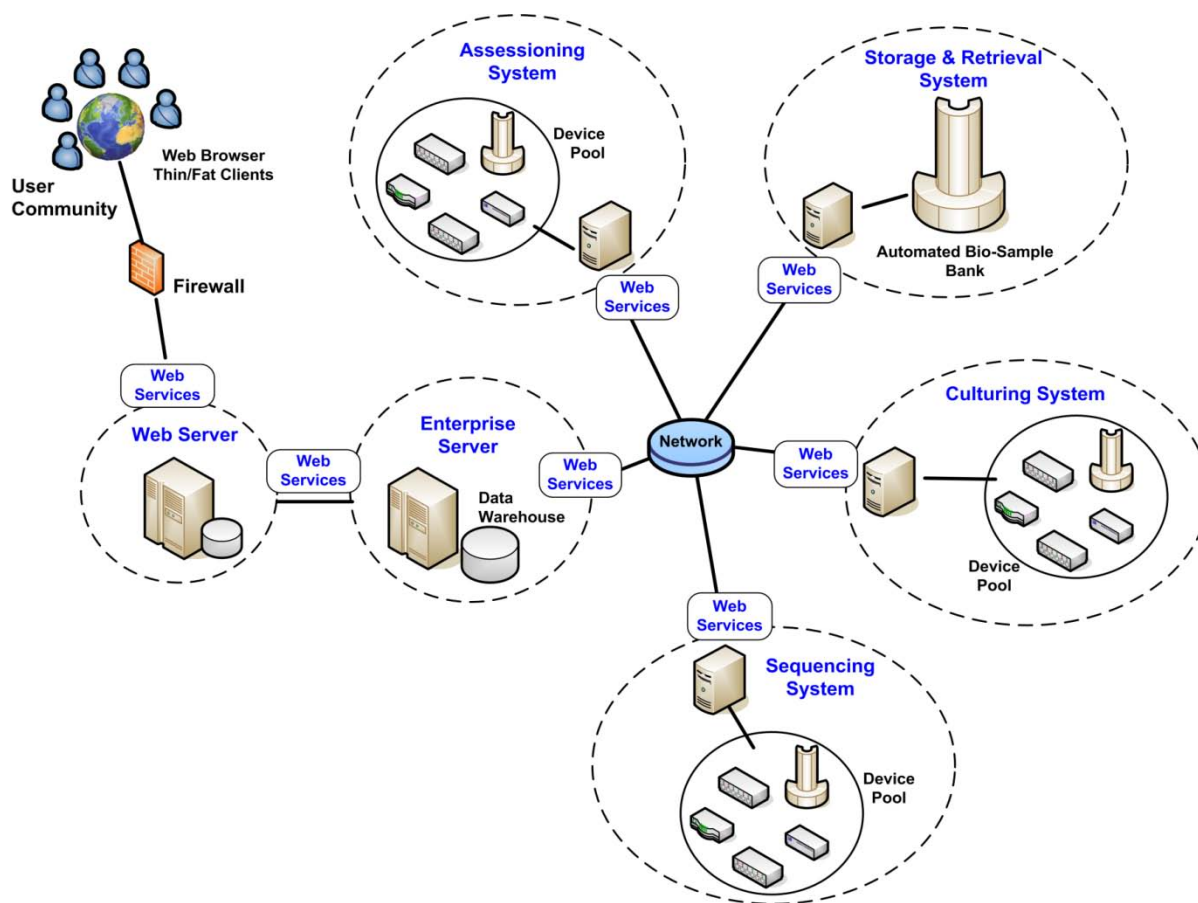

Figure 1. Web service-based Systems Integration

Limiting work cell cross-communications to the use of well-defined, published Web services and standards such as XML and SOAP will provide us with the much needed plug-and-play flexibility and multi-vendor interoperability.
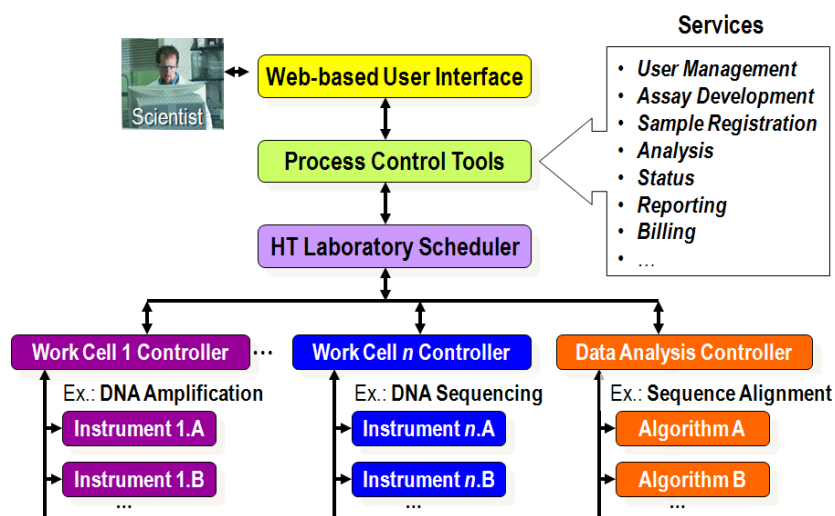

Figure 2. Service-Oriented, Layered System Architecture

LIMS Integration

The LIMS product of choice for the HTLN is StarLIMS (www.starlims.com). StarLIMS is a web-based LIMS that natively provides a set of well-defined Web services to simplify integration with 3rd party products and systems. The HTLN StarLIMS will be installed on a networked, hardware and software-mirrored Dell PowerEdge-based server, running the 64-bit versions of Windows Server 2003 R2 and Microsoft SQL Server 2005.

Data Representation and Exchange

Similar to the device/work cell control scheme outlined above, data exchange among work cells and StarLIMS will be implemented via Web services. The data representation standard of choice for HTLN is XML. To simplify data exchange and transformations, XML-formatted data is always preferred to proprietary data structures.

UNIVERSITY OF CALIFORNIA, LOS ANGELES
REQUEST FOR PROPOSAL

ISSUE DATE: **March 6, 2008**

RFP NO: **MMLG0-2008-005**

DUE DATE: **April 11, 2008**

TIME: **3:00 PM (Pacific Daylight Time)**

PROPOSALS MUST BE RECEIVED BY THE DUE DATE AND TIME TO BE CONSIDERED.

Genotyping System

for

University of California, Los Angeles

TABLE OF CONTENTS

1   RFP PURPOSE AND BACKGROUND

1.1   Organizational Context

Founded as the state's first and only land grant institution in 1868, the University of California is a system of 10 campuses with approximately 180,000 undergraduate and graduate students. The official research arm of the State of California, UC has five medical schools, four law schools and the nation's largest continuing education program. It also manages three national laboratories that are engaged in energy and environmental research and approximately 130,000 acres of natural habitat in California for research, teaching and outreach activities. The University's fundamental mission is teaching, research and public service. Additional information regarding the University of California can be found at http://www.ucop.edu.

The Los Angeles campus of UC (UCLA) is a public research university and a member of the Association of American Universities with an enrollment of 25,715 undergraduates and 12,883 graduate students and 3,326 faculty members. UCLA is located in Westwood Village in Los Angeles, about 5 miles from the Pacific Ocean and consists of 174 buildings on 419 acres. Academic programs include a College of Letters and Science, 11 professional schools offering 118 undergraduate and 200 post graduate degrees.

1.2   RFP Purpose

This Request for Proposal (RFP) is an invitation to qualified Bidders to submit a Proposal to furnish a Genotyping System to the University in accordance with the specifications, terms and conditions set forth in this (RFP).

2   INSTRUCTIONS TO BIDDERS

2.1   Issuing Office and University Contact

This RFP is being issued by the Office of UCLA Campus Procurement, which is the only office authorized to change, modify, clarify, etc., the provisions of this RFP and to award any contract(s) resulting from this RFP.

The single point of contact for administrative and technical issues regarding this RFP is:

      Lee Gordon
      UCLA Campus Procurement
      Phone: (310) 794-6016
      Fax: (310) 794-8020
      E-mail: lgordon@finance.ucla.edu

2.2   RFP Schedule

Listed below are the key action times/dates for this RFP. If the University determines that it is necessary to change any of the dates as indicated below, an addendum to the RFP will be issued.

EVENT	TIME (PDT)	DATE
Release of Request for Proposal		March 6, 2008
Deadline for Notice of Intent to Submit Proposal & participate in the Project Meeting	3:00 PM	March 11, 2008
Mandatory Project Meeting	10:00 AM	March 14, 2008
Deadline for RFP Questions	3:00 PM	March 20, 2008
Answers to Questions	3:00 PM	March 26, 2008
Proposals Due	3:00 PM	April 11, 2008
Estimated Contract Award		May 2008

2.3   Notice of Intent to Submit Proposal

Bidders should send a completed Notice of Intent to Submit Proposal (see Section 10.1) by the due date set forth in Section 2.2 above to the attention of the University contact person listed in Section 2.1 above in order to receive any subsequent RFP notifications, addenda, updates, etc.

2.4   Mandatory Project Meeting

Bidders are required to participate in the Project Meeting to obtain the information necessary for formulating and submitting complete and thorough proposals. Bidders who do not participate in the meeting will not be qualified to submit an RFP. **Bidders must complete and return Attachment 10.4 to confirm their participation.**

This **mandatory project meeting** gives all parties the opportunity to exchange information prior to the actual submission of proposals. The teleconference provides a forum by which interested parties have equal access to relevant RFP information prior to the final proposal submission.

Participation will consist of calling into a teleconference that will be hosted by University administrative and technical associates. **The names of your representatives who will be calling in and participating must be delivered to the Point of Contact by 3:00 PM PDT, March 11th, 2008**. It is anticipated that the duration of the teleconference will be approximately two hours.

> Date: Friday, March 14, 2008
> Time: 10:00 AM to 12:00 PM (PDT)
> Dial-In
> Number: 310.794.9375

2.5   Proposal Receipt

One (1) signed original, Three (3) hardcopies and two (2) discs of the proposal must be delivered to the following address prior to the proposal submission deadline specified in the RFP Schedule (see Section 2.2).

> **Lee Gordon**
> **UCLA Campus Procurement**
> **10920 Wilshire Boulevard, Suite 650**
> **Los Angeles, CA 90024**

Proposals shall be in a sealed envelope marked:

> **Name of Bidder**
> **RFP Number: MMLG0-2008-005**
> **Proposal Due Date and Time**

No telephone, email, or facsimile proposals will be accepted. Proposals received after the time for closing will be returned to the Bidder unopened.

2.6   Bidder Questions

Bidders are expected to exercise their best professional independent judgment in analyzing the requirements of this RFP to ascertain whether additional clarification is necessary or desirable before responding. If there are any discrepancies in, or omissions to the RFP, or if there are any questions as to any information provided in the RFP or by any other source, a request must be submitted via email by using the Bidder Inquiry Form as set forth in Section 8 for clarification, interpretation or correction by the date stipulated in Section 2.2 above. Such inquires must be directed to the contact person listed in Section 2.1 above.

2.7   Restriction on Communications

Except for the designated contact person listed in Section 2.1 above, Bidders are not permitted to communicate with the University staff regarding this solicitation during the period between the RFP issue date and the announcement of awards, except during:

- The course of a Bidders' conference, if conducted;
- Oral presentations and site visits, if conducted.

If a Bidder is found to be in violation of this provision, the University reserves the right to reject its proposal.

2.8   Proposal Format and Required Submittals

Bidders are to provide a written proposal detailing the full scope of the project and its proposed activities.

Proposals shall be submitted in the following format. Proposals in any other format will be considered informal and will be rejected. Conditional proposals will not be considered. An individual authorized to extend a formal proposal must sign all proposals. If the bidder fails to provide any of the following information, with the exception of the mandatory proposal certification, the University may at its sole option, ask the Bidder to provide the missing information or evaluate the proposal without the missing information.

Proposals must include all of the elements listed, be clearly indexed and assembled (in accordance with the numbers and order listed below) and reference the corresponding RFP Sections and paragraphs.

1.   Table of Contents - Proposals must include a table of contents with page numbers covering all parts including exhibits and addenda, with sufficient detail to facilitate easy reference to all requested information.

2.   Signed Proposal Certification - RFP Section 9.

3.   Executive Summary - This section should present an introduction and general description of the company's background, nature of business activities, and experience relevant to this RFP. This section should also provide a statement of the Bidder's understanding of the major objectives of the RFP. The overview should contain a brief summary of the Bidder's approach to fulfilling the requirements, including a description of the salient features and distinctive merits of the proposed products and/or services. The summary should be readily understandable by non-technical persons at the management level and should be no more than three pages in length.

4.   Response to the Project Scope of Work – RFP Section 3. The response must identify all products and/or services Bidder proposes to fulfill the stated requirements. Proposals must follow the specified format as closely as possible for ease of evaluation by addressing the requirements in the order and manner presented, in sufficient detail to describe how the Bidder's products and/or services meets the stated requirements. If the Bidder cannot perform any part of the work as specified, this must be clearly stated in the proposal.   Responses should indicate any deficiencies, enhancements, or other differences that exist between the offered products and/or services and those which the University has described in its specifications.

5.   Cost Proposal – Bidders must provide a cost proposal in the form and format specified in the RFP Section 7.   Please provide the cost information in a separate sealed envelope clearly marked "Cost Proposal" and include with the proposal copy marked "Original" only (do not include in any other copies of the proposal).

6.   Terms and Conditions Acceptance - Indicate acceptance/compliance with all items in RFP Section 6.

7.   University of California Business Information Form – RFP Sections 2.16 and 10.2.

8.   Audited Annual Report or Financial Statements for the past two (2) years – Reference RFP Section 4.

9.   Experience List – Reference RFP Section 4.

10.   Proposed Subcontract List – Reference RFP Section 6.14

11.   Supplementary Information and Additional Comments, if desired.

2.9   RFP Exceptions

- ***The University's Mandatory Requirements set forth in Section 2.18 – No exceptions are allowed.***

- Technical Exceptions: Bidder shall clearly describe any and all deviations in its Proposal from the functional requirements stated in this RFP and also describe any services that could be made by the Bidder to satisfy those requirements.

- General Exceptions: Bidder shall also clearly state its objections, exceptions, or alternatives to the general (non-technical) requirements stated in this RFP. If the Bidder has no general exceptions to present, this fact should be stated in the Proposal.

- Bidders are cautioned that if the University is unwilling or unable to approve a request for exception to the RFP requirements and Bidder does not withdraw the request, the proposal will be deemed to be non-responsive and ineligible for contract award.

2.10   Proposal Validity Period and Costs

All proposals shall remain valid and subject to University contract award for a minimum of ninety (90) days following the RFP due date.   All costs incurred in the preparation and submission of proposals and related documentation, including Bidder presentation to UCLA, will be borne by the Bidder. No modification of submitted proposals will be permitted in any form. No proposal shall be withdrawn once it is submitted.

2.11   Bidder Representation

Bidder represents that it is an independent contractor and bonded in its state of business and conducts business according to the requirements of such state, and Bidder desires to provide the products and/or services on the terms and conditions provided herein. By submitting a proposal, Bidder also represents that it has:

- Read and completely understands the RFP and associated documents.
- Based the proposal upon the requirements described in the RFP.

2.12   Simplicity of Preparation

Proposals should be prepared simply and economically, providing a straightforward, concise description of the Bidder's capability to satisfy the requirements of the RFP. Emphasis should be on completeness and clarity of content.   Special bindings, color displays, etc., are not desired.   Promotional materials are especially discouraged.

2.13   Complete Proposals

All proposals must be full and complete at the time of proposal submittal.

2.14   Specifications

Bidders are expected to meet or exceed the specifications in their entirety. Each proposal shall be in accordance with the RFP. If products and/or services as proposed do not comply with the RFP as written, Bidder shall attach to bid proposal a complete detailed itemization and explanation for each and every deviation or variation from these specifications. Absence of any such itemization and explanation shall be understood to mean that Bidder proposed to meet all details of these specifications.

2.15   Amendments to RFP before Due Date

No individual is authorized to amend any part of this RFP in any respect, by an oral statement, or to make any representation of interpretation in conflict with provision of this RFP prior to the proposal submission date. However, if necessary, supplemental information in addenda form will be provided to all prospective Bidders who have received this RFP from the Office of UCLA Campus Procurement.   Failure of any Bidder to receive such addenda shall not relieve the Bidder from any obligation under their proposal as submitted.   All addenda so issued shall become part of this RFP.

2.16   University of California Business Information Form

All Bidders must complete the University of California Business Information Form (see Section 10.2) (UAA101 1/92) and return it as part of the proposal.

2.17   Contract Negotiation

Any contract awarded pursuant to this RFP will incorporate the requirements, specifications, terms, and conditions contained in this RFP, as well as the contents of the Bidder's proposal as accepted by the University. The University reserves the right to negotiate modification of the terms and conditions contained in the RFP/Proposal with the apparent successful bidder prior to the execution of a contract to ensure a satisfactory contract, provided that such modifications would not materially alter the nature of the competition. If the parties are unable to reach agreement, the University may go to the next lowest responsive and responsible Bidder.

2.18   Rejection of Proposals

The University reserves the right to reject proposals which are non-responsive, including, without limitation, proposals which contain the following defects:

- Late or incomplete proposals;
- Failure to meet the Minimum Qualifications of Bidders contained in Section 4 in the RFP;
- Failure to follow the Proposal Format and Required Submittals contained in Section 2 in the RFP;
- Make any exceptions to the Mandatory Requirements in Section 3.
- Failure to sign the proposal;
- Proposals which contain false or misleading statements, or which provide references which do not support an attribute or condition claimed by the Bidder.
- Proof of collusion among Bidders, in which case all proposals involved in the collusive action will be rejected;
- Noncompliance with applicable law, unauthorized additions or deletions, conditional proposals, incomplete proposals, or irregularities of any kind which may tend to make the proposal incomplete, indefinite or ambiguous as to its meaning;
- Provisions reserving the right to accept or reject an award or to enter into a contract containing terms and conditions that are contrary to those in the solicitation.

2.19   Errors and Omissions

If Bidder discovers any discrepancy, ambiguity, error, or omission in this RFP or any related documents, the Bidder should notify the University immediately and request clarification or correction. Any such errors or omissions if verified by the University, will be corrected by written addendum to the RFP. If the RFP contains an error or omission known, or that reasonably should have been known to the Bidder, and if the Bidder fails to notify the University of such error or omission prior to the date fixed for submission of proposals, and if the Bidder is subsequently awarded the contract, the Bidder shall not be entitled to additional compensation or time for performance by reason of the error or omission, or its later correction.

2.20   Clarification of Proposals

Prior to contract award, the University may, at its sole discretion, seek clarification from any Bidder regarding proposal information and may do so without notification to any other Bidder.

3   SCOPE OF WORK

The development of an integrated, automated system that performs the sequence of steps listed in sections 3.1 through 3.4.

3.1   PCR

Using Qiagen's OneStep RT-PCR kit.

96 well plate - 10ul reactions;
7ul of master mix (buffer, dNTPs, water)
1ul RNA sample
2ul of primers
10ul
example – one 96 well plate (1 sample): would make a Xul of master mix add Xul of RNA, dispense 8ul of RNA/master mix into plate and then dispense 2ul of primers. OR dispense 7ul of master mix only, then 1ul of RNA, then 2ul of primers. Seal plate, spin down, and put into a thermal cycler for amplification.

384 well plate – 5ul reactions (preferred scenario):
3ul of master mix (buffer, dNTPs, water)
1ul RNA sample
1ul of primers
5ul
example – one 384 well plate (up to 4 samples): would make a Xul master mix add Xul RNA, dispense 4ul of RNA/master mix into each quadrant and then dispense 1ul of primers into each quadrant. OR dispense 3ul of master mix only into all 4 quadrants, then 1ul of RNA, then 1ul of primers. Seal plate, spin down, and put into a thermal cycler for amplification.

Notes: Master mix will be in a 96 well plate. Also, RNA samples will be in 96 well plates and primers will be in 96 well plates. Would likely need cooled deck stations for master mixes (enzymes and RNA).

3.2   PCR Cleanup

Currently using Agencourt's AMPure system.

96 well plate – 10ul reactions:
10ul of PCR products are cleaned with 18ul of beads, washed two times with 200ul of 70% ethanol, and eluted in up to 40ul of TE buffer.

384 well plate – 5ul reactions (preferred scenario):
5ul of PCR products are cleaned with 9ul of beads, washed two times with 30ul of 70% ethanol, and eluted in up to 30ul of TE buffer.

Requirements: System must be capable of pumping 70% ethanol while keeping magnetic beads agitated.

3.3   Sequencing Reaction

Using ABI's Big Dye Terminator sequencing system on the ABI 3730.
384 well plate – 5ul reactions
3ul master mix (BDT, buffer, water)
1ul PCR product
1ul primer
5ul

Example – one 384 well plate (up to two samples of forward and reverse coverage). Would make up Xul of master mix, dispense 3ul of master mix into each quadrant, then 1ul of PCR product into designated quadrant, and 1ul of primer into designated quadrant. Seal plate, spin down, and put into a thermal cycler for sequencing.

Requirements: System must be capable of handling multiple milliliters of master mix in a tube. System may need cooled deck stations for master mixes (enzymes).

3.4   Sequencing Cleanup

Currently using Agencourt's CleanSEQ system.

384 well plate – 5ul reactions:
5ul sequencing reactions are cleaned with 5ul of beads + 14.3ul of 85% ethanol, then washed two times with 30ul of 85% ethanol and eluted in up to 30ul of water.

Requirements: System must be capable of pumping 85% ethanol while keeping magnetic beads agitated.

3.5   IT Requirements for Automated Laboratory

These IT requirements must also be included in the development of the integrated, automated system.

The purpose of this section is to briefly outline the general IT requirements for the automated laboratory instrumentation for UCLA's High-Throughput Influenza Laboratory (HTLN).

Objective

From an IT and systems integration perspective, the HTLN will be based on a Service-Oriented Architecture (SOA) approach. With respect to laboratory automation hardware and software, this means that every automated system (e.g., sample accessioning, sample storage and retrieval, culturing, sequencing, and data analysis; Figure 1) will be treated as a service provider.

3.6   Implementation of Web Services

Rather than providing low-level , platform and programming language-dependent control interfaces for individual devices (e.g., an ActiveX control for each device), HTLN automation software and hardware

suppliers are expected to provide macro-level (i.e., work cell-level), Web service-based control interfaces (Figure 1). Functional and logical grouping and abstraction of instrumentation in form of Web services will allow us to treat any sub-system/work cell as a black box with well-defined inputs and outputs for material and data.

By using a Web services based approach to instrument control and communications, we not only provide a solid foundation for true plug-and-play integration of multi-vendor systems, but we also provide vendors with absolute implementation freedom.

LANL intends to provide the selected automation vendor(s) with the necessary WSDL-compliant (Web Service Description Language) interface definitions. It is important to note that these Web service definitions will be aimed at macro-level (i.e., work cell-level) controls rather than low-level controls. For example, a use case analysis of an automated sample storage and retrieval system would yield that such a system basically provides two services—1) Sample Check In, and 2) Sample Check Out. Internally, however, each of these services may require a certain sequence of steps, such as moving a robotic arm, scanning barcodes, etc. Rather than exposing low-level commands as Web services and requiring the service request to follow a certain device-specific call sequence, providing a macro level command (i.e., service) such as "CheckIN <plate id> <location>" is much preferred.

For automated systems and/or system components that do not natively provide a Web service-based interface, but which are considered to be a service provider, the vendor(s) would be expected to provide a Web service based "wrapper" around their legacy control interface(s).

3.7   Instrument Scheduling and Resource Management

As illustrated in Figure 1 and Figure 2, each work cell will have its own, dedicated, networked front end controller. Each work cell's front end controller is expected to provide and implement a certain set of Web services (based upon a prior work cell-level use case analysis). As mentioned earlier, LANL will provide the equipment vendor with the necessary WSDL interface definitions (and associated XML based data structures).

While resource sharing across work cells may become necessary, it is important to note that for simplicity reasons each device or subsystem will be managed by only one dedicated, networked, controller. For example, to obtain access to a resource that is part of another work cell, a work cell controller would have to send a resource allocation request to the resource's local controller via its published Web service interface.

Limiting work cell cross-communications to the use of well-defined, published Web services and standards such as XML and SOAP will provide us with the much needed plug-and-play flexibility and multi-vendor interoperability.

3.8   LIMS Integration

The LIMS product of choice for the HTLN is StarLIMS (www.starlims.com). StarLIMS is a web-based LIMS that natively provides a set of well-defined Web services to simplify integration with 3rd party products and systems. The HTLN StarLIMS will be installed on a networked, hardware and software-mirrored Dell

PowerEdge-based server, running the 64 bit versions of Windows Server 2003 R2 and Microsoft SQL Server 2005.

3.9   Data Representation and Exchange

Similar to the device/work cell control scheme outlined above, data exchange among work cells and StarLIMS will be implemented via Web services. The data representation standard of choice for HTLN is XML. To simplify data exchange and transformations, XML-formatted data is always preferred to proprietary data structures.

4   BIDDER QUALIFICATION

4.1   Minimum Qualifications of Bidders

The University believes that the Bidder's experience, financial capability, expertise of personnel, and related factors are important in assessing the Bidder's potential to successfully fulfill the requirements defined in this RFP. Accordingly, Bidders must provide the information below and meet minimum qualification requirements as determined by the University in order to be considered for award.

Bidder must be able to demonstrate a record of past financial stability and positive indicators for future performance.

Bidders are to submit an audited annual report or audit annual financial statement for the past two (2) years for which such reports or statements are available (including all notes), or tax returns for the two (2) most recent tax years; The University also reserves the right to obtain Dun & Bradstreet reports, or similar independent reports, for further indications of the Vendor's ability to provide the products and services specified in this RFP.

Bidders are requested to provide their Dun & Bradstreet company number.

- Bidders must have the ability to obtain the necessary insurance (ref.: Article 17 of the enclosed University of California Terms and Conditions of Purchase, Appendix A);

Bidders must meet the following minimum requirements, and show proof of the following in their RFP proposal. A response of "NO" to any of the following questions will disqualify the Bidder's Proposal.

- Vendor has been in the laboratory system integration system design business for
  a minimum of five (5) years.   YES [   ]   NO [   ]

- Vendor has all required licenses in order to complete the services specified in this RFP.

                                                    YES [   ]   NO [   ]

- Vendor must have at least five (5) years experience within the last 10 years working in a University environment.

<div align="center">YES [   ]   NO [   ]</div>

Vendor must include in their RFP response a minimum of five (5) and a maximum of eight (8) references from businesses where vendor has provided a similar scope of services for a similar sized account. References must include the company name, contact, position, telephone number, fax number, and if available, the E-Mail address, of a contact person.

<div align="center">YES [   ]   NO [   ]</div>

In addition to the information required above, University may request additional information either from the Bidder or others, to verify the Bidder's ability to successfully meet the requirements of this RFP.

4.2   Vendor's Background and Experience

Bidders are required to include the following information in their proposals:

        A.   Length of time in business, type of license and technical qualifications of the firm.

        B.   Number of employees in company.

        C.   An organizational chart of your company.

        D.   Description of a minimum of three (3) similar projects successfully completed within the past five (5) years.

        E.   Advantages your company offers that differentiate your company from your competitors

<div align="center">

5   METHOD OF AWARD

</div>

5.1   Proposal Evaluation Method

The contract resulting from this RFP, if any, shall be awarded to the responsible Bidder whose proposal is determined to be the most advantageous to the University; taking into consideration the evaluation factors set forth in this solicitation.

Eligible proposals will be examined by a University evaluation team and scored using a quality points system. The intent of the evaluation process is to determine, through application of uniform criteria, how effectively the proposed products and/or services satisfy the University's requirements. In addition to information provided in the proposal, the evaluation team may

utilize site visits, or may request oral presentations, additional material, information, or references from the Bidders and others. The evaluation team will assign quality point scores to each proposal using the criteria listed below. The points assigned by each evaluator will be added together to determine the total quality points for each proposal. The total quality points to determine will then be divided by the total proposed cost to determine the proposal offering the lowest cost per point.

5.2   Evaluation Criteria

Quality points will be awarded to each proposal based on the following:

- Quality, clarity and responsiveness of the Bid proposals in conformance with instructions, conditions and the RFP format
- Ability of the Bidder to meet the required Scope of Work
- Ability of the Bidder to meet the University needs and goals
- Bidder's background, experience and core competency in aligning with project requirements
- Bidder's ability to fulfill all project requirements and meet University schedules
- Completeness of employee listings, staffing of sites and pricing
- Management experience, financial capability and reference checks
- Staffing, supervisory experience, technical skills and service responsiveness
- Training programs

Proposals must meet or exceed a pre-determined minimum number of quality points in order to be considered for award. Any proposals that are found to be administratively or technically non-responsive are subject to disqualification

5.3   Selection of Award Winner

The responsive and responsible Bidder offering the lowest cost per quality point will be recommended for award. Should the Bidder with the proposal offering lowest cost per quality point refuse or fail to accept the tendered purchase contract, the award may be made successively to the Bidder with the second lowest cost per quality point, or then to the third in the event of further failure to accept.

Exceptions taken in proposals, or irregularities therein, may be negotiated with or corrected by the Bidder involved provided that, in the judgment of the University, such action will not negate fair competition and will permit proper comparative evaluation of proposals submitted. The University's waiver of an immaterial deviation or defect shall in no way modify the RFP documents or excuse the Bidder from full compliance with the Request for Proposal specifications in the event the contract is awarded to that Bidder.

The University reserves the right to accept or reject any or all proposals, make more than one award, or no award, as the best interests of the University may appear. Any contract award resulting from this RFP will incorporate the terms, conditions, and requirements contained in the RFP, as well all relevant provisions of the related proposal.

5.4   Bidder Reference Checks

The evaluation committee reserves the right to contact, interview and evaluate the Bidder's references; contact any Bidder to clarify any response; contact and interview any current users of Bidder's services; solicit information from any available source concerning any aspect of a proposal; and seek and review any other information deemed pertinent to the evaluation process.

5.5   Contract Award in Best Interest of University

The University reserves the right to accept or reject proposals on each item separately or as a whole, to make one award, multiple awards or no award, to reject any or all proposals without penalty, to waive any informalities or irregularities therein, and to contract as the best interest of the University may require in order to obtain the products and/or services which best meets the needs of the University, as expressed in this RFP. The University reserves the right to negotiate the modification of, terms and conditions with the bidder offering the best value to the University, in conjunction with the award criteria contained herein, prior to the execution of a contract to ensure a satisfactory contract.

6   TERMS AND CONDITIONS

6.1   Appendix "A"

The terms and conditions governing any contract resulting from this RFP shall be pursuant to those contained in this document as well as those contained in the University of California Standard Terms and Conditions of Purchase Appendix A (see Section 10.3).

6.2   University of California Employees

All proposals must indicate any/all known University of California employees and/or near relatives who hold a position in your organization or have been engaged as a consultant for your organization within the last two years.   Also indicate any known University of California employees or near relatives that own or control more than a ten percent (10%) interest in your organization. If there are none, so state.

6.3   Conflict of Interest

By submitting a proposal in response to this RFP, the Bidder certifies that no officer or employee of University has any financial interest, direct or indirect, in the bidding firm. Any question, which may arise during the performance of this agreement regarding a possible conflict of interest, shall be referred to University for adjudication.   Bidder(s) awarded a contract pursuant to this RFP shall not hire or contract with any officer or employee of University or any member of their immediate family to perform any service covered by the contract.

6.4   Ethics

Bidder shall exercise extreme care and due diligence to prevent any action or conditions which could result in conflict with the best interest of the University.

Throughout the term of any agreement resulting from the RFP, Bidder shall not accept any employment or engage in any work which creates a conflict of interest with the University or in any way compromises the work to be performed under this RFP or any agreement resulting from this RFP. Bidder and its employees will not offer gifts, entertainment, payment, loans, or other gratuities or consideration to University employees, their families, other Contractors, subcontractors, or other third (3rd) parties for the purpose of influencing such persons to act contrary to the University's interest or for personal gain. Bidder shall immediately notify the University of any and all such violations of this clause upon becoming aware of such violations.

6.5   University's Right to Reject or Modify

Selection of a proposal does not mean that all aspects of the proposal(s) are acceptable to the University. The University reserves the right to negotiate the modification of the proposal terms and conditions prior to the execution of a contract, to ensure a satisfactory procurement.

6.6   Project Changes

Any changes requested by the contractor must be submitted in writing to the University for review and require written approval by UCLA Campus Procurement. Change Orders (CO's) will be based on pricing provided in response to this RFP.

6.7   Form of Agreement

The contents of this RFP, RFP Addenda, and the proposal document of the successful Bidder(s) shall become contractual obligations as part of the contract if acquisition action ensues. Failure of successful Bidder(s) to accept these obligations in a contractual agreement shall result in cancellation of award. The University reserves the right to negotiate provisions in addition to those stipulated in this RFP or proposed by Bidder for the purpose of obtaining the best possible contract.

6.8   Performance Standard

All work performed shall be the highest quality in every respect and shall conform to the highest standards of the industry.

6.9   Marketing References

The successful Bidder shall be prohibited from making any reference to the University, in any literature, promotional material, brochures, or sales presentations without the express written consent of a University officer with the appropriate delegated authority in accordance with University of California policy regarding the use of the University's Names, Seals, and Trademarks (see http://www.adminvc.ucla.edu/appm/public/app_0110_0.html)

UCLA Policy 110 Use of the University's Names, Seals, and Trademarks.url

6.10   Disclosure of Records

All bids, supporting materials, and related documentation will become the property of University. This Request for Proposal, together with copies of all documents pertaining to any award, if issued, shall be kept for a period of five years from date of contract expiration or termination and made part of a file or record which shall be open to public inspection. If the response contains any trade secrets that should not be disclosed to the public or used by University for any purpose other than evaluation of your approach, the top of each sheet of such information must be marked with the following legend:

<div align="center">"CONFIDENTIAL INFORMATION"</div>

 All information submitted as part of the bid must be open to public inspection (except items marked as trade secrets and considered trade secrets under the California Public Records Act) after the award has been made.

Note: Pricing is not considered trade secret information and will be subject to disclosure.

Should a request be made of University for information that has been designated as confidential by the Bidder and on the basis of that designation, University denies the request for information; the Bidder shall be responsible for all legal costs necessary to defend such action if the denial is challenged in a court of law.

6.11   Proprietary Information

Any restrictions on the use of data contained in a proposal must be clearly stated in the proposal itself. Proprietary information submitted in response to the RFP will be handled in accordance with applicable University of California procurement regulations and the Public Records Act. University and Bidders agree that any computer software programs, documentation, or similar products developed by Bidders under the resulting contract constitute work(s) made for hire under the Federal Copyright Act of 1976 ("The Act") and University shall own all rights to such work product including without limitation, the copyright therein throughout the world. To the extent said products do not constitute works made for hire under the Act, Bidders agrees to assign all rights, title, and interest in said products to the University.

6.12   Audit Requirement

Any agreement resulting from this RFP shall be subject to an examination and audit by the University and the State of California for a period of three (3) years after final payment. The examination and audit shall be confined to those matters connected with the performance of the agreement, including but not limited to the costs of administering the agreement.

6.13   Insurance Requirements

Vendor shall furnish a certificate of insurance acceptable to the University. All certificates shall name the Regents of the University of California as an additional insured. The certificate must be submitted to the UCLA Purchasing Department prior to the commencement of services. Bidder must review attached Appendix A for insurance requirement guidelines. Certificates of insurance should be delivered to the Point of Contact listed in Section 2.1 when and if an award is made.

6.14   Subcontractors

Vendor shall not assign work or services resulting from this RFP to sub-contractors without prior, specific, case-by-case written approval.

6.15   Marketing References

Vendor shall be prohibited from making any reference to UCLA, in any literature, promotional material, brochures, or sales presentations without the express written consent of UCLA.

6.16   Cost Reasonableness

Bidder certifies that its cost proposal quoted in the proposal submitted in response to this RFP are the lowest rate(s) quoted to any other University, governmental agency, other educational customer or similar customer for similar products and/or services provided.

6.17   Confidentiality

Bidders, their officers, agents, employees, and consultants shall hold in confidence any information or materials identified as proprietary and/or confidential to UCLA or to any third party, to which Bidders may have access to in the course of performing its obligations under this RFP. Bidders shall not disclose or authorize disclosure to others, or use for its own benefit, such confidential information or materials without the express written consent of UCLA or any third party owner. This obligation for non-disclosure shall survive this RFP and continue until such confidential information or materials are otherwise legally obtained or placed in the public domain.

6.18   Payment Terms

Acceptable invoices shall be paid net 30 days.

6.19   Terms Included and Order of Precedence

In submitting a proposal in response to this RFP, Bidder acknowledges that this RFP, including all appendices and attachments, and including service, financial and program specifications and terms and conditions will be incorporated in its entirety in any award issued in response to this RFP. Other documents to be incorporated in the resulting Agreement shall include the Bidder's entire proposal, including all brochures, attachments and supplementary information. However,

in the event of any conflict between the RFP and the Bidder's proposal, the terms of this RFP shall control, and govern any matter set forth therein that is not explicitly modified, added or deleted by the provisions of the subsequent Agreement.

7   COST PROPOSAL

Please read carefully – costs must be provided in a separate sealed envelope

Cost proposal must include sufficient itemization to enable the University to evaluate the rate structure of Bidder's proposal. If the cost proposal is not complete and/or do not clearly indicate all rate elements, proposals may be rejected by the University.

Bidders are to provide the required cost information in a separate sealed envelope clearly marked "Cost Proposal" and enclose it with the RFP copy that is marked "original".

All proposed products and/or services necessary to meet RFP requirements and perform the scope of work must be clearly identified in the cost proposal.   Bidders must include any additional items and cost information which may be relevant to the proposal.   Unless otherwise specified, prices will be F.O.B. University of California, Los Angeles.

Cost Proposals must include sufficient itemization to enable University to evaluate the reasonableness and fairness of all cost elements.   Please quote your lowest price for the proposed products and services. Any deviation from the specifications must be identified and fully described. The Cost Proposal must clearly indicate if bidder is unable to provide one or more of the required item(s).

No charges for other incidental costs will be allowed over and above those prices quoted per this RFP. The right is reserved to accept or reject quotation on each item separately, or as a whole, and to waive any irregularities in a quotation.

UCLA payment terms are normally net 30 days, following delivery and acceptance of the products and/or services. Bidders should quote any available discounts offered for prompt payment (less than 30 days).

Pricing must be valid for a minimum of 90 days from the closing date for submitting proposals.

Pricing will not be subject to any increase after the closing date for submission of proposals.

8   BIDDER INQUIRY FORM

Bidders should use this form to submit questions regarding this RFP. University will provide a complete list of questions received along with the University's responses to all Bidders who participate.   Questions will be listed without reference to the source.

RFP NUMBER: MMLG0-2008-005
ISSUE DATE: March 6, 2008
PROPOSALS SOLICITED FOR: Genotyping System
DEADLINE FOR RFP QUESTIONS: March 20, 2008   3:00 PM (PDT)

SUBMIT QUESTIONS TO: lgordon@finance.ucla.edu

Company Name:
Company Representative:
E-mail Address:
Question(s)

9   BIDDER CERTIFICATION PAGE

RFP NUMBER： MMLG0-2008-005
ISSUE DATE: March 6, 2008
PROPOSALS SOLICITED FOR: Genotyping System
PROPOSAL DUE DATE & TIME: April 11, 2008   3:00 PM (PDT)

PROPOSALS MUST BE RECEIVED ON OR BEFORE THE DUE DATE SHOWN ABOVE. LATE PROPOSALS WILL NOT BE ACCEPTED.

          <u>SUBMIT PROPOSALS TO:</u>
Lee Gordon
UCLA Campus Procurement
10920 Wilshire Blvd., Suite 650
Los Angeles, CA 90024

BIDDER MUST SIGN WHERE INDICATED BELOW AND SUBMIT THIS FORM AS THE COVER PAGE IN ORDER TO VALIDATE ITS PROPOSAL
This proposal is in response to the above-said Request for Proposal. This proposal consists of the Bidder's Certification and Exceptions to the RFP documents. Bidder agrees to perform in accordance with all provisions of the RFP documents and addenda thereto, except as may be specifically stated in its proposal, at the commission rate(s) set forth herein. Bidder agrees that this proposal is a firm commission rate(s) offer to the University, which cannot be withdrawn for the number of days set forth in Section 2.9 of this RFP from and after the proposal due date .   **Bidder certifies that it has thoroughly examined and fully understands all of the provisions of the RFP and the conditions of the contract documents, as well as any addenda issued prior to the due date; that it has carefully reviewed and fully supports the accuracy of its proposal; has satisfied itself as to the nature and location of all work, the requirements of the contract and all other matters which may in any way affect performance or the cost thereof; and that the University shall not be responsible for any errors or omissions on the part of the undersigned in preparing this proposal.**   **If awarded a contract, Bidder agrees to execute the contract and deliver it to the University within 15 calendar days of such award, along with any required documents.**
Signature of Bidder's Representative (Attach a Power of Attorney)   Date
Printed name of Bidder's Representative
Title of Bidder's Representative
Bidder's Full Company Name, Address, Tel No., Fax No., E-mail Address
Bidder's Taxpayer I.D. Number

EXCEPTIONS TO SECTION 6 OF THE RFP

Bidder is aware that exceptions to the University's terms and conditions will be negatively weighted as part of the University's evaluation of its proposal.

_____   No exceptions proposed. All terms and conditions in Section 6 are accepted.

_____   Exceptions proposed. The following specific modifications to the terms and conditions in Section 6 are proposed:

10   ATTACHMENTS

10.1   NOTICE OF INTENT TO SUBMIT PROPOSAL

10.2   BUSINESS INFORMATION FORM

10.3   APPENDIX A

10.4   PROJECT MEETING

10.1   NOTICE OF INTENT TO SUBMIT PROPOSAL

RFP NUMBER: MMLG0-2008-005
ISSUE DATE: March 6, 2008
PROPOSALS SOLICITED FOR: Genotyping System
DEADLINE FOR THIS NOTICE: March 11, 2008   3:00 PM (PDT)
SUBMIT THIS NOTICE via E-MAIL TO: lgordon@finance.ucla.edu or FAX TO: LEE GORDON @ 310-794-8020

PLEASE PROVIDE THE FOLLOWING INFORMATION FOR THE PERSON WHO WILL BE THE BIDDER'S PRINCIPAL CONTACT FOR MATTERS REGARDING THE ABOVE RFP.
Company Name and Address
Signature of Bidder's Representative
Printed name of Bidder's Representative
Title
Direct Tel. No.
Main Tel. No.
Main Fax No.
Email Address
License Number
Tax ID Number

10.2   BUSINESS INFORMATION FORM

(Please refer to the attached BIF)

10.3   APPENDIX A
UCLA Standard Terms and Conditions of Purchase

ARTICLE 1 - The materials, supplies or services covered by this order shall be furnished by Seller subject to all the terms and conditions set forth in this order including the following, which Seller, in accepting this order, agrees to be bound by and to comply with in all particulars and no other terms or conditions shall be binding upon the parties unless hereafter accepted by them in writing. Written acceptance or shipment of all or any portion of the materials or supplies, or the performance of all or any portion of the services, covered by this order shall constitute unqualified acceptance of all its terms and conditions. The terms of any proposal referred to in this order are included and made a part of the order only to the extent it specifies the materials, supplies, or services ordered, the price therefore, and the delivery thereof, and then only to the extent that such terms are consistent with the terms and conditions of this order.

ARTICLE 2 - INSPECTION. The services, materials and supplies furnished shall be exactly as specified in this order free from all defects in Seller's performance, design, workmanship and materials, and, except as otherwise provided in this order, shall be subject to inspection and test by University at all times and places. If, prior to final acceptance, any services and any materials and supplies furnished therewith are found to be incomplete, or not as specified, University may reject them, require Seller to correct them without charge, or require delivery of such materials, supplies, or services at a reduction in price which is equitable under the circumstances. If Seller is unable or refuses to correct such items within a time deemed reasonable by University, University may terminate the order in whole or in part. Seller shall bear all risks as to rejected services and, in addition to any costs for which Seller may become liable to University under other provisions of this order, shall reimburse University for all transportation costs, other related costs incurred, or payments to Seller in accordance with the terms of this order for unaccepted services and materials and supplies incidental thereto. Notwithstanding final acceptance and payment, Seller shall be liable for latent defects, fraud or such gross mistakes as amount to fraud.

ARTICLE 3 - CHANGES. University may make changes within the general scope of this order in drawings and specifications for specially manufactured supplies, place of delivery, method of shipment or packing of the order by giving notice to Seller and subsequently confirming such changes in writing. If such changes affect the cost of or the time required for performance of this order, an equitable adjustment in the price or delivery or both shall be made. No change by Seller shall be allowed without written approval of University. Any claim of Seller for an adjustment under this Article must be made in writing within thirty (30) days from the date of receipt by Seller of notification of such change unless University waives this condition in writing. Nothing in this Article shall excuse Seller from proceeding with performance of the order as changed hereunder.

ARTICLE 4 - TERMINATION
A. University may, by written notice stating the extent and effective date, cancel and/or terminate this order for convenience in whole or in part, at any time. University shall pay Seller as full compensation for performance until such termination:

(1) the unit or pro rata order price for the performed and accepted portion; and
(2) a reasonable amount, not otherwise recoverable from other sources by Seller as approved by University, with respect to the unperformed or unaccepted portion of this order, provided compensation hereunder shall in no event exceed the total order price.

B. University may by written notice terminate this order for Seller's default, in whole or in part, at any time, if Seller refuses or fails to comply with the provisions of this order, or so fails to make progress as to endanger performance and does not cure such failure within a reasonable period of time, or fails to perform the services within the time specified or any written UNEX thereof. In such event, University may purchase or otherwise secure services and, except as otherwise provided herein, Seller shall be liable to University for any excess costs occasioned University thereby. If, after notice of termination for default, University determines that the Seller was not in default or that the failure to perform this order was due to causes beyond the control and without the fault or negligence of Seller (including, but not restricted to,

acts of God or of the public enemy, acts of University, acts of Government, fires, floods, epidemics, quarantine restrictions, strikes, freight embargoes, unusually severe weather, and delays of a subcontractor or Contractor due to such causes and without the fault or negligence of the subcontractor or Contractor), termination shall be deemed for the convenience of University, unless University shall determine that the services covered by this order were obtainable by Seller from other sources in sufficient time to meet the required performance schedule.

C. If University determines that Seller has been delayed in the work due to causes beyond the control and without the fault or negligence of Seller, University may extend the time for completion of the work called for by this order, when promptly applied for in writing by Seller; any UNEX granted shall be effective only if given in writing. If such delay is due to failure of University, not caused or contributed to by Seller, to perform services or deliver property in accordance with the terms of the order, the time and price of the order shall be subject to change under the Changes Article. Sole remedy of Seller in event of delay by failure of University to perform shall, however, be limited to any money actually and necessarily expended in the work during the period of delay, solely by reason of the delay. No allowance will be made for anticipated profits.

D. The rights and remedies of University provided in this Article shall not be exclusive and are in addition to any other rights and remedies provided by law or under this order. E. As used in this Article, the word "Seller" includes Seller and its subcontractors at any tier.

ARTICLE 5 - LIABILITY FOR UNIVERSITY - FURNISHED PROPERTY. Seller assumes complete liability for any tooling, articles or material furnished by University to Seller in connection with this order and Seller agrees to pay for all such tooling, articles or material damaged or spoiled by it or not otherwise accounted for to University's satisfaction. The furnishing to Seller of any tooling, articles, or material in connection with this order shall not, unless otherwise expressly provided, be construed to vest title thereto in Seller.

ARTICLE 6 - TITLE. Title to the material and supplies purchased hereunder shall pass directly from Seller to University at the f.o.b. point shown, or as otherwise specified in this order, subject to the right of University to reject upon inspection.

ARTICLE 7 - PAYMENT, EXTRA CHARGES, DRAFTS. Seller shall be paid, upon submission of acceptable invoices, for materials and supplies delivered and accepted or services rendered and accepted. University will not pay cartage, shipping, packaging or boxing expenses, unless specified in this order. Drafts will not be honored. Invoices must be accompanied by shipping documents or photocopies of such, if transportation is payable and charged as a separate item.

ARTICLE 8 - CHARACTER OF SERVICES. Seller, as an independent contractor, shall furnish all equipment, personnel and material sufficient to provide the services expeditiously and efficiently during as many hours per shift and shifts per week and at such locations as the University may so require and designate.

ARTICLE 9 - FORCED, CONVICT, AND INDENTURED LABOR
A. By accepting this order, Seller hereby certifies that no foreign-made equipment, materials, or supplies furnished to the University pursuant to this order will be produced in whole or in part by forced labor, convict labor, or indentured labor under penal sanction.

B. Any Seller contracting with the University who knew or should have known that the foreign-made equipment, materials, or supplies furnished to the University were produced in whole or in part by forced labor, convict labor, or indentured labor under penal sanction, when entering into a contract pursuant to the above, may have any or all of the following sanctions imposed:

(1.) The contract under which the prohibited equipment, materials, or supplies were provided may be voided at the option of the University.

(2.) Seller may be removed from consideration for University contracts for a period not to exceed 360 days.

ARTICLE 10 - INDEMNITY.
A. General. Seller shall defend, indemnify, and hold harmless University, its officers, employees, and agents, from and against all losses, expenses (including attorneys' fees), damages, and liabilities of any kind resulting from or arising out of this agreement and/or Seller's performance hereunder, provided such losses, expenses, damages and liabilities are due or claimed to be due to the negligent or willful acts or omissions of Seller, its officers, employees, agents, subcontractors, or anyone directly or indirectly employed by them, or any person or persons under Seller's direction and control.

B. Proprietary Rights. Seller shall indemnify, defend, and hold harmless University, its officers, agents, and employees against all losses, damages, liabilities, costs, and expenses (including but not limited to attorneys' fees) resulting from any judgment or proceeding in which it is determined, or any settlement agreement arising out of the allegation, that Seller's furnishing or supplying University with parts, goods, components, programs, practices, or methods under this order or University's use of such parts, goods, components, programs, practices, or methods supplied by Seller under this order constitutes an infringement of any patent, copyright, trademark, trade name, trade secret, or other proprietary or contractual right of any third party. The foregoing shall not apply unless University has informed Seller as soon as practicable of the suit or action alleging such infringement. Seller shall not settle such suit or action without the consent of University. University retains the right to participate in the defense against any such suit or action.

C. Products. Seller shall fully indemnify, defend, and hold harmless University from and against any and all claim, action, and liability, for injury, death, and property damage, arising out of the dispensing or use of any of Seller's product provided under authorized University orders. In addition to the liability imposed by law on the Seller for damage or injury (including death) to persons or property by reason of the negligence, willful acts or omissions, or strict liability of the Seller or his agents, which liability is not impaired or otherwise affected hereby, the Seller hereby assumes liability for and agrees to save University harmless and indemnify it from every expense, liability or payment by reason of any damage or injury (including death) to persons or property suffered or claimed to have been suffered through any act or omission of the Seller.

The University agrees to provide Seller with prompt notice of any such claims and to permit Seller to defend any claim or suit, and that it will cooperate fully in such defense.

ARTICLE 11 - DECLARED VALUATION OF SHIPMENTS. Except as otherwise provided on the face of this order, all shipments by Seller under this order for University's account shall be made at the maximum declared value applicable to the lowest transportation rate or classification and the bill of lading shall so note.

ARTICLE 12 - WARRANTY. Seller warrants that the work performed and/or product furnished under this order shall be free from defects in title, shall conform in all respects to the terms of the order, and if not specified in the order shall comply with all applicable standards in the industry, and shall be free from defects in design, material and workmanship. Seller's warranty shall be effective for a warranty period of twenty-four (24) months from Acceptance Date, however, that any redesign, recoding or reprogramming performed in the warranty period shall be re-warranted for twelve (12) months from completion of such redesign, recoding or reprogramming. If Seller does not commence to cure any defect or nonconformity immediately after notification, University may take steps to cure the defect or nonconformity and Seller shall be liable to University for the actual costs of University's remedial action. Failure of University to discover defects or non-conformities shall in no way relieve Seller of its responsibility during the term of this Order and for warranty period described herein to immediately make such modifications as are required to minimize delay and/or damage to the work. The rights and remedies so provided are in addition to and do not limit any rights afforded to University by any other article of this order. Such warranties will be effective notwithstanding prior inspection and/or acceptance of the services or supplies by the University.

ARTICLE 13 - ASSIGNMENT AND SUBCONTRACTING. This order is assignable by University. Except as to any payment due hereunder, this order may not be assigned or subcontracted by Seller without written approval of University. In case such consent is given, it shall not relieve Seller from any of the obligations of this Agreement and any transferee or subcontractor shall be considered the agent of Seller and, as between the parties hereto, Seller shall be and remain liable as if no such transfer or subcontracting had been made.

ARTICLE 14 - EQUAL OPPORTUNITY AFFIRMATIVE ACTION. Seller shall not maintain or provide racially segregated facilities for employees at any establishment under its control. Seller agrees to adhere to the requirements set forth in Executive Orders 11246 and 11375, and with respect to activities occurring in the State of California, to the California Fair Employment and Housing Act (Government Code section 12900 et seq.). Expressly, Seller shall not discriminate against any employee or applicant for employment because of race, color, religion, sex, national origin, ancestry, medical condition (as defined by California Code section 12925f]), marital status, age, physical and mental handicap in regard to any position for which the employee or applicant for employment is qualified, or because he or she is a disabled veteran or veteran of the Vietnam era. Seller shall further specifically undertake affirmative action regarding the hiring, promotion and treatment of minority group persons, women, the handicapped, and disabled veterans and veterans of the Vietnam era. Seller shall communicate this policy in both English and Spanish to all persons concerned within its company, with outside recruiting services, and the minority community at large. Seller shall provide the University on request a breakdown of its labor force by groups, specifying the above characteristics within job categories, and shall discuss with the University its policies and practices relating to its affirmative action programs.

ARTICLE 15 - The clauses contained in the following paragraphs of the Federal Acquisition Regulations are incorporated by reference. The full text is available upon request:

FAR 52.222-04 Contract Work Hours and Safety Standards Act
FAR 52.222-26 Equal Opportunity
FAR 52.223-02 Clean Air and Water (If order exceeds $100,000)

ARTICLE 16 - WORK ON UNIVERSITY OR GOVERNMENT PREMISES. If Seller's work under this order involves performance by Seller at University or United States Government owned sites or facilities, the following provisions shall apply:

A. Liens. Seller agrees that at any time upon request of University he will submit a sworn statement setting forth the work performed or material furnished by subcontractors, Contractors and materialmen, and the amount due and to become due to each, and that before the final payment called for hereunder, will if requested, submit to University a complete set of vouchers showing what payments have been made for materials and labor used in connection with the work called for hereunder. Seller shall: (1) Indemnify and hold harmless University from all claims, demands, causes of action or suits, of whatever nature, arising out of the services, labor and materials furnished by Seller or its subcontractors under this order, and from all laborers', materialmen's and mechanics' liens upon the real property upon which the work is located or any other property of University;

(2) Promptly notify University in writing, of any such claims, demands, causes of action, or suits brought to its attention. Seller shall forward with such notification copies of all pertinent papers received by Seller with respect to any such claims, demands, causes of action or suits and, at the request of University shall do all things and execute and deliver all appropriate documents and assignments in favor of University of all Seller's rights and claims growing out of such asserted claims as will enable University to protect its interest by litigation or otherwise. The final payment shall not be made until Seller, if required, shall deliver to University a complete release of all liens arising out of this order, or receipts in full in lieu thereof, as University may require, and if required in either case, an affidavit that as far as it has knowledge or information, the receipts include all the labor and materials for which a lien could be filed; but Seller may, if any subcontractor refuses to furnish a release or receipt in full, furnish a bond satisfactory to University to indemnify it against any claim by lien or otherwise. If any lien or claim remains unsatisfied after all

payments are made, Seller shall refund to University all monies that the latter may be compelled to pay in discharging such lien or claim, including all costs and reasonable attorneys' fees.

B. Cleaning Up. Seller shall at all times keep University premises where the work is performed and adjoining premises free from accumulations of waste material or rubbish caused by its employees or work of any of its subcontractors, and, at the completion of the work; shall remove all rubbish from and about the building and all its and its subcontractors' tools, scaffolding, and surplus materials, and shall leave the work "broom clean" or its equivalent, unless more exactly specified. In case of dispute between Seller and the subcontractors employed on or about the structure or structures upon which the work is to be done, as herein provided, as to responsibility for the removal of the rubbish, or in case the same be not promptly removed as herein required, University may remove the rubbish and charge the cost to Seller.

C. Employees. Seller shall not employ on the work any unfit person or anyone not skilled in the work assigned to him or her, and shall devote only its best-qualified personnel to work under this order. Should University deem anyone employed on the work incompetent or unfit for his or her duties and so inform Seller, Seller shall immediately remove such person from work under this order and he or she shall not again, without written permission of University, be assigned to work under this order. It is understood that if employees of University shall perform any acts for the purpose of discharging the responsibility undertaken by the Seller in this Article 15, whether requested to perform such acts by the Seller or not, such employees of the University while performing such acts shall be considered the agents and servants of the Seller subject to the exclusive control of the Seller.

D. Safety, Health and Fire Protection. Seller shall take all reasonable precautions in the performance of the work under this order to protect the health and safety of employees and members of the public and to minimize danger from all hazards to life and property, and shall comply with all health, safety, and fire protection regulations and requirements (including reporting requirements) of University. In the event that Seller fails to comply with said regulations or requirements of University, University may, without prejudice to any other legal or contractual rights of University, issue an order stopping all or any part of the work; thereafter a start order for resumption of work may be issued at the discretion of the University. Seller shall make no claim for UNEX of time or for compensation or damages by reason of or in connection with such work stoppage.

The safety of all persons employed by Seller and its subcontractors on University premises, or any other person who enters upon University premises for reasons relating to this order, shall be the sole responsibility of Seller. Seller shall at all times maintain good order among its employees and shall not employ on the work any unfit person or anyone not skilled in the work assigned to him or her. Seller shall confine its employees and all other persons who come onto University's premises at Seller's request or for reasons relating to this order and its equipment to that portion of University's premises where the work under this order is to be performed or to roads leading to and from such work sites, and to any other area which University may permit Seller to use. Seller shall take all reasonable measures and precautions at all times to prevent injuries to or the death of any of its employees or any other person who enters upon University premises. Such measures and precautions shall include, but shall not be limited to, all safeguards and warnings necessary to protect workers and others against any conditions on Owner's premises which could be dangerous and to prevent accidents of any kind whenever work is being performed in proximity to any moving or operating machinery, equipment or facilities, whether such machinery, equipment or facilities are the property of or are being operated by, the Seller, its subcontractors, the University or other persons. To the extent compliance is required, Seller shall comply with all University safety rules and regulations when on University premises.

ARTICLE 17 - INSURANCE
Seller shall defend, indemnify, and hold the University, its officers, employees, and agents harmless from and against any and all liability, loss, expense (including reasonable attorneys' fees), or claims for injury or damages that are caused by or result from the negligent or intentional acts or omissions of Seller, its officers, agents, or employees. Seller, at its sole cost and expense, shall insure its activities in connection with the work under this order and obtain, keep in force, and maintain insurance as follows:

A. Comprehensive or Commercial Form General Liability Insurance (contractual liability included) with limits as follows:

Each Occurrence $ 2,000,000.00
Products/Completed Operations Aggregate $5,000,000.00
Medical Expense $10,000.00
Personal and Advertising Injury $2,000,000.00
Fire Damage $100,000.00
General Aggregate (Not applicable to the Comprehensive Form) $5,000,000.00

If the above insurance is written on a claims-made form, it shall continue for three years following termination of this Agreement. The insurance shall have a retroactive date of placement prior to or coinciding with the effective date of this Agreement.

B. Business Automobile Liability Insurance for owned, scheduled, non-owned, or hired automobiles with a combined single limit not less than One Million dollars ($1,000,000.00) per occurrence. (REQUIRED ONLY IF SELLER DRIVES ON UNIVERSITY PREMISES IN THE
COURSE OF PERFORMING WORK FOR UNIVERSITY.)

C. Professional Liability Insurance with a limit of One Million ($1,000,0000.00) dollars per occurrence with an aggregate of not less than One Million ($1,000,000.00) dollars. If this insurance is written on a claims-made form, it shall continue for three years following termination of this Agreement. The insurance shall have a retroactive date of placement prior to or coinciding with the effective date of this Agreement.

D. Workers' Compensation: Meet the statuary requirements.

It is understood that the coverage and limits referred to under a., b., and c. above shall not in any way limit the liability of Seller. Seller shall furnish the University with certificates of insurance evidencing compliance with all requirements prior to commencing work under this Agreement. Such certificates shall:

(1) Provide for thirty (30)-days advance written notice to the University of any modification, change, or cancellation of any of the above insurance coverage.
(2) Indicate that The Regents of the University of California has been endorsed as an additional insured for the coverage referred to under a. and b. This provision shall only apply in proportion to and to the extent of the negligent acts or omissions of Seller, its officers, agents, or employees.
(3) Include a provision that the coverage will be primary and will not participate with nor be excess over any valid and collectible insurance or program of self-insurance carried or maintained by the University.

ARTICLE 18 - PERMITS. Seller agrees to procure all necessary permits or licenses and abide by all applicable laws, regulations and ordinances of the United States and of the state, territory and political subdivision in which the work under this order is performed. Seller shall be liable for all damages and shall indemnify and save University harmless from and against all damages and liability which may arise out of failure of Seller to secure and pay for any such licenses or permits or to comply fully with any and all applicable laws, ordinances and regulations.

ARTICLE 19 - COOPERATION. Seller and its subcontractors, if any, shall cooperate with University and other vendors and contractors on the premises and shall so carry on their work that other cooperating vendors and contractors shall not be hindered, delayed or interfered with in the progress of their work, and so that all of such work shall be a finished and complete job of its kind.

ARTICLE 20 - WAIVER OF DEFAULT. Any failure of University at any time, or from time to time, to enforce or require the strict keeping and performance by Seller of any of the terms or conditions of this order shall not constitute a waiver by University of a breach of any such terms or conditions and shall not affect or impair such terms or conditions in any way, or the right of University at any time to avail itself of such remedies as it may have for any such breach or breaches of such terms or conditions.

ARTICLE 21 - TAXES. Seller shall pay all contributions, taxes and premiums payable under federal, state and local laws measured upon the payroll of employees engaged in the performance of work under this order, and all applicable sales, use, excise, transportation, privilege, occupational and other taxes applicable to materials and supplies furnished or work performed hereunder and shall save University harmless from liability for any such contributions, premiums, and taxes.

ARTICLE 22 - OTHER APPLICABLE LAWS. Any provision required to be included in a contract of this type by any applicable and valid federal, state or local law, ordinance, rule or regulations shall be deemed to be incorporated herein.

ARTICLE 23 - GOVERNING LAW. The law of the State of California shall control this Appendix and any document to which it is appended.

Rev. 8/99

10.4   Project Meeting Notification

REQUEST FOR PROPOSAL
MANDATORY PROJECT MEETING

PROJECT NAME:

GENOTYPING   SYSTEM

Request for Proposal Number
MMLG0-2008-005

Date:	**Friday, March 14, 2008**
Time:	**10:00 AM (PDT)**
Dial-In	
Number:	**310.794.9375**

SIGN AND FAX THIS COVER SHEET TO: ATTENTION: Lee Gordon
Fax Number: 310.794.8020

Company:	Name:
Date:	Signature:
Telephone Number:	Title:
Number of people participating:	E-mail: